ECOLOGICAL AND POLLUTION STUDIES OF THE BRITISH CRAYFISH

by

Christopher Charles Mees, B.Sc.

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ABSTRACT

The ecology of *Austropotamobius pallipes* (Lereboullet) from Markfield Quarry and the River Leen was described. Studies included:

1. Biology.

(i) Timing of life cycle events. They related to ambient conditions, especially temperature.

(ii) Fecundity. Individual fecundity increased with female size. Population fecundity related to population density.

(iii) Local distribution. This related to hide availability. Gross water quality affected the distribution of the river crayfish.

2. Population dynamics.

(i) Population size/density. That of the Quarry was greater, and related to hide availability. Seasonal variations in population size were temperature dependent.

(ii) Population structure.

- size structure varied between populations due to collection techniques. It varied seasonally due to recruitment and differential catchability of certain sub-populations.
- sex ratios varied seasonally due to reduced foraging by ovigerous females.
- disease and damage occurred for all sizes/sexes. *Thelohania contejeanii* was absent from Markfield Quarry but increased in the Leen during the study period.

3. Growth.

(i) At moulting. Sexual differences were absent for the absolute increment, but males grew quicker due to greater moult frequencies. Growth rates of river animals were greatest

due to a longer growing season and smaller population density.

(ii) Relative growth of body parts. No consistent population differences occurred, but of significance were chelae and rostrum sizes. Sexual dimorphism occurred for certain variables, notably the chelae and abdomen width.

The effects of cadmium and Lindane upon *A. pallipes* were examined. Studies included:

1. Survival. Juveniles were 10 times more sensitive than adults. Lindane was the most toxic.
2. Uptake and depuration. Cadmium accumulated chiefly in the gills. Some evidence for its translocation to other tissues was found. No sexual differences occurred. Lindane accumulated chiefly in the hepatopancreas. Evidence for its translocation and depuration was shown. No sexual differences occurred.
3. Tissue oxygen consumption. Both toxicants caused a depression. Recovery occurred with time.

The results were related to the levels of toxicant in Midlands waters.

GENERAL INTRODUCTION

Austropotamobius pallipes pallipes (Lereboullet) is the only crayfish species indigenous to Britain (Thomas and Ingle 1971; Gledhill *et. al.* 1976). It has increasingly been stated that this species deserves more attention, and that study of its basic ecology, which until recently was virtually unknown, is essential (Morriarty, 1971; Thomas and Ingle, 1971; Ingle, 1976; Holdich *et. al.*, 1978; Sutcliffe, 1978; Rhodes and Holdich, 1979; Pratten, 1980; Rhodes, 1981). The reasons for this relatively recent upsurge of interest are due to increasing demands for crayfish as luxury food items (Richards and Fuke, 1977). Associated with this are false reports that *A. pallipes* is now virtually extinct from Britain (eg Anonymous, 1976), which has meant that foreign crayfish species have been introduced to meet the demand. In Europe similar introductions have resulted in the decimation of indigenous populations due to the introduction of the crayfish plague, *Aphanomyces astaci*, in the mid-Nineteenth century. There is a fear that this may reach the British Isles, and there is therefore a need for detailed studies of the ecology of *A. pallipes*, before it is too late!

Of the recent studies which have resulted, there are reports on the distribution of *A. pallipes*, in the British Isles (Jay and Holdich, 1981), field studies of a population in the south of England, (Pratten, 1980) and one in the north of England, (Bowler and Brown, 1978; Brewis, 1978; Brown, 1979; Brown and Bowler, 1978; Brewis and Bowler, 1982) and a laboratory study of several Midlands populations (Rhodes and Holdich, 1979; Rhodes, 1980; Rhodes, 1981). Thus information is wanting as to the natural ecology of Midlands populations, which require attention as they form a geographic intermediate between those already studied. Results

are therefore given in Part I of this thesis which relate to two Midlands populations, one in the River Leen, in Nottinghamshire, and one in Markfield Quarry, in Leicestershire. They are two very different habitats, one being an 'open' ecosystem, and is a fast flowing, shallow stream, whilst the other is a 'closed' ecosystem, consisting of a relatively deep water-filled quarry with no inlets or outlets.

Part II reports the results of a laboratory study into the effects of two pollutants, a heavy metal, cadmium, and a pesticide, Lindane, upon *A. pallipes*. To date, only the effects of varying pH have been examined in relation to the British freshwater crayfish (Jay and Holdich, 1976), and no other effects of pollution have been examined. The importance of such studies, however, must not be underestimated. Any study of the ecology, or population dynamics, of a particular animal needs also to examine the possible factors and processes which may affect the fluctuation and regulation of the population. Pollution is increasingly becoming one such factor and may be responsible for population disappearances. This is an important factor to determine when arguments arise such as the disputed arrival of the crayfish plague in Britain (Karlsson, 1978; Bowler, 1979).

In the U.S.A. it has been recognized that pollution has destroyed certain crayfish populations (Hobbs and Hall, 1975). Here in Britain, pollution has also been implicated in the decline of certain populations (e.g Duffield, 1933) and it has been stated that both heavy metals (Davies, 1964) and pesticides (Bowler, 1979), may affect crayfish populations. These suppositions, however, are unsubstantiated and thus information of a more positive nature

is now presented. These are particularly relevant to the Trent River system which has become increasingly polluted since the Industrial Revolution and from which populations of crayfish have been eliminated (Roberts, pers. comm.).

PART I

THE ECOLOGY OF *A. PALLIPES* IN THE MIDLANDS

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INTRODUCTION

There are some 500 crayfish species throughout the world, (Karlsson, 1977) which fall into two major groups - the Astacidae of the northern hemisphere which inhabit Europe, Asia, and North America, and the Parastacidae of the southern hemisphere, found in Australasia, Tasmania, New Zealand, the Fiji Islands, Madagascar and South America (Chiddester, 1912). In some countries such as Madagascar and New Guinea they form an essential source of protein in the diet, whilst in Europe they are considered a delicacy, and are sold as a luxury food item (Karlsson, 1977).

As previously stated there is only one crayfish species indigenous to the British Isles, i.e. *Austropotamobius pallipes* Lereboullet (Decapoda; Astacidae). It has been largely neglected in the past, because unlike in Europe, where crayfish are greatly appreciated gastronomically, it has not been much eaten in this country. Thus it has passed almost unnoticed since the nineteenth century when Huxley (1896) wrote his book entitled "The Crayfish". Today, however, there is an upsurge of interest which has arisen chiefly from commercial concern, and from the introduction of foreign crayfish species into Britain (Richards and Fuke, 1977).

The two major concerns of British crayfish biologists are as to whether *A. pallipes* could form a potential food resource, (e.g. Holdich *et. al.*, 1978; Goddard and Holdich, 1979; Rhodes, and Holdich, 1979), and secondly to determine the basic ecology of this species. This is necessary in the face of possible competition

from the introduction of foreign crayfish species, and even erradication of *A. pallipes* due to the introduction of the crayfish plague which is associated with these species (e.g. see Bowler, 1979). It is with these two concerns in mind that the biology of *A. pallipes* has been studied. An examination of the ecology of crayfish from two widely differing habitats is presented, and aspects of their population dynamics and growth rates are also discussed, including the factors which affect the local distributions of the two populations.

A. pallipes has three to five subspecies (Gledhill *et. al*, 1976). It is found in many countries in Europe, including Austria, Corsica, France, Germany, Spain, Yugoslavia and Britain, (see Rhodes, 1980 for review) but with the exception of the latter, they are in competition with other crayfish species, i.e. *Astacus astacus*, *Astacus leptodactylus*, and *Austropotamobius torrentium*. This has meant that *A. pallipes* is confined to small streams in Europe, which is in direct contrast to the situation observed in Britain where they occupy a much wider ecological range of habitats (from fast flowing streams to large standing water bodies, often with silty bottoms) and are widely distributed and abundant (Huxley, 1896; Davies 1964; Thomas and Ingle, 1971; Morriarty, 1972; Holdich *et. al.* , 1978; Jay and Holdich 1981). Arguments to the contrary which have arisen in the past (Anonymous, 1976; Karlsson, 1978) have been proven to be totally false (Jay and Holdich, 1981). Thus, in view of the potential dangers to our native species, it is essential that examination of its basic ecology be undertaken.

The role of crayfish in the energetics of freshwater communities is not precisely understood. However, in view of their relative

abundance in some areas, this role must be substantial. It has been suggested that crayfish play an important role in preventing massive growths of weeds, and in maintaining the biological balance of waterways (Abrahamsson, 1966; Spitzy, 1972). Since they have been observed to eat detrital matter and dead fish, (see 1.3.) they must also play an important role in the breakdown of organic matter, thus preventing eutrophication. In addition, they also enter the food web as prey for other animals such as perch, pike, trout, ducks and herons (see 1.3.). Thus it may be seen that should crayfish be erradicated from British waters, the result could also involve quite substantial changes of other plant and animal species, with the water quality or communities no longer being suitable.

The potential threat to the British crayfish arises from the possible introduction of the crayfish plague. It is a fungal disease, *Aphonomyces astaci* , which attacks the non calcified parts of the cuticle. This disease is believed to be indigenous to the North American continent, but was introduced into Italy in 1860. It spread rapidly through France and central Europe, reaching Russia by the turn of the century. In 1907 it reached Sweden, and was reported in Finland in 1971 (Unestam, 1972). All the European crayfish including *Austropotamobius*, and *Astacus*, are "oversusceptible" to the disease, whilst the American species have developed an immunity, and it is thought that they may also carry the disease without being affected (Unestam, 1969). Extermination of all crayfish from lakes and rivers in Europe has resulted wherever the disease has been present, and particularly affected have been *Astacus astacus* and *A. leptodactylus* (Abrahamsson, 1972b; Laurent,

1972; Spitzy, 1972; Westman, 1972, 1974; Brinck,1974; Furst, 1977).

Studies of the population biology of *A. pallipes* in conjunction with details of factors affecting both local and nationwide distribution effectively form a baseline survey of the crayfish in Britain. Thus any decline in the stocks of crayfish may be readily assessed, and hopefully the rightful causes of the decline elucidated. Variations in population numbers of crayfish in Britain have been recorded both on an annual basis, (e.g. Thomas and Ingle, 1971; Morriarty, 1972; Brown, 1979) and on a yearly, and more long term basis (Duffield, 1933, 1936; Pixell-Goodrich, 1956). Annual variations are due to variations in the catchability of individuals which result from decreased activity in colder weather, or migration into deeper waters. Some behavioural variations affecting catchability may also occur, for example when a female becomes ovigerous. The yearly and long term variations are not so easy to explain. Reasons speculated for such variations and population declines include disease (fungal, bacterial, and protozoan infections) predation, pollution, drought, migration, overfishing, and extremes of temperature from summer to winter (Duffield, 1933, 1936; Pixell-Goodrich, 1956; Davies, 1964; Vey and Vego, 1972; Karlsson, 1977; Holdich *et. al.* 1978). It has even been suggested that the crayfish plague has already affected British populations (Richards and Fuke, 1977; Karlsson, 1978) although there was no evidence to support this (Holdich *et. al.* 1978; Bowler, 1979) until recently when plague was suspected as being a possible cause of mass mortalities of crayfish in southern England (Alderman, pers. comm.).

It is also important to study the population biology of *A. pallipes* in order to evaluate whether or not they could form

a viable alternative food resource to the import of foreign crayfish species. It has been suggested that immediate demand could be met by cropping natural populations, and future demand by the culture of *A. pallipes*, (Holdich *et. al.*, 1978). Rhodes and Holdich (1979) have also examined the first steps in assessing the commercial exploitation potential of *A. pallipes*. Certainly the demand for crayfish in Europe is high, and even in 1977 they would fetch £8 - £12 per kilo (Karlsson, 1977). However, Spitzy (1972) reports that the existence of *A. pallipes* in Austrian waters is economically unimportant, and generally throughout Europe the trend has been to seek a foreign alternative.

Orconectes limosus, *Procambarus clarkii*, and *Pacifastacus leniusculus* have all been introduced into Europe from the U.S.A., but *P. clarkii* being a warm water species has not been successful (Karlsson, 1977; Holdich *et. al*, 1978). Of the remaining two, *P. leniusculus* is the preferred species (Abrahamsson, 1972b; Goldman, 1972; Spitzy, 1972; Brinck, 1974, 1976; Furst, 1977). It is very similar to *Astacus astacus*, but grows faster, reproduces sooner, has more eggs per female, weighs more (Abrahamsson, 1972b) and has "the same excellent taste" (Karlsson, 1977). Introduction into Britain began in 1976 (Anonymous, 1976) into a number of privately owned ponds, lakes, and fish farms. The fear that escapes may occur into the general network of waterways appears justified and are reported to have occurred in Scotland (Jay and Holdich, 1981). Direct introductions of *P. leniusculus* into rivers containing existing populations of *A. pallipes* have even been reported, in the south of England (Hogger, 1982). This will undoubtably have unknown, and possibly disasterous effects on the ecology of the

native species. Section 14 of the 1981 Wildlife and Countryside act now makes provision for the M.A.F.F. to licence introductions of alien animals into the wild, but evidently this has come too late, although it is possible that the crayfish introduced so far may not establish themselves. Certainly previous introductions of *A. astacus*, *A. leptodactylus* and *P. clarkii* appear to have failed to become established (Jay and Holdich, 1981; Hogger, 1982).

In order to overcome the problem of introducing the crayfish plague along with *P. leniusculus*, Sweden has banned the import of live animals and developed a culture programme to provide 'seed' for restocking purposes (Karlsson, 1977). Thus, the Simontorp Company in Sweden is now one of the largest suppliers of juvenile crayfish, although more British suppliers are now establishing themselves (e.g. Anonymous, 1976; Richards and Fuke, 1977; Brown, 1982) thus exacerbating the potential dangers to the native crayfish in Britain.

In addition to the need for assessment of population sizes, in order to determine the potential viability of *A. pallipes* as a commercial concern, growth rates and relative growth rates of meat containing regions (i.e. chelae and abdomen) need to be examined. Comparisons may then be drawn with species such as *P. leniusculus* with which it will have to compete. The meat yield of *A. pallipes* has been studied, and compares favourably (Rhodes and Holdich, 1979) but obviously of vital importance in any commercial venture is the timescale involved to achieve the yields required, and for crayfish to reach a marketable size. Thus results are made available in this thesis which relate to the growth and relative growth of *A. pallipes*. Similarly results are presented for the other topics

discussed above. They relate to two Midlands populations. Although the body of knowledge relating to *A. pallipes* in Britain has increased in recent years, such studies are still wanting for Midlands populations for which only laboratory studies have been conducted in the past. These populations, in the River Leen (Nottinghamshire), and Markfield Quarry (Leicestershire) are themselves from very different habitats, and also form a geographical intermediate between those populations studied previously.

CHAPTER 1

A DESCRIPTION OF THE POPULATION STUDY AREAS

1.1 THE RIVER LEEN

1.1(i) INTRODUCTION

The River Leen is a relatively small tributary of the River Trent, situated in a lowland part of the Nottinghamshire region, and north of the City of Nottingham where a succession of different rock types occur forming North-South belts. It lies roughly in the divide between two of these rock types, namely the Permian-magnesian limestone, and the Bunter sandstone (King, 1966) and runs north to south from Kirkby forest to join the River Trent to the west of Trent Bridge. Permian-magnesian limestone beds change to sandy facies in some areas (Taylor, 1966) and indeed, the substrate of the river is variable along its length (see 3.3). In its uppermost reaches the river is divided into two branches known as the East and West branches of the Leen.

The catchment area of the River Leen is approximately 31,000 acres of which 50% is fully urbanized (T.R.A., 1966), this being chiefly the lower reaches of the river where it flows through the connurbation of Nottingham (see Fig. 1.1). In its upper reaches however, the River Leen flows through arable farm land (Bennett-Jones and Jones, 1966; Edwards, 1966) and woodland and it is in this area that the study population is located. Historically the River Leen has been utilized variously by the people of Nottingham and the surrounding towns. In 1696 the first recorded public water supplies came from a pumping station on the Leen near to its confluence with the Trent, and later, in the nineteenth century, another water works was opened at Basford at a point free from

contamination by the town's sewers. However, by the mid nineteenth century all the major rivers in the Nottingham area, including the Leen, had become too polluted for use as public water supplies, so boreholes were sunk into the Bunter sandstone and pumping stations were opened at Basford (1857), Bestwood (1871) and Papplewick (1883) (Adams, 1966).

Sources of pollution in the River Leen have been various and increasing industrialization and population growth have inevitably resulted in a deterioration of the water quality. Sewage effluents enter the river from points of habitation all along its length and there are also several water reclamation works. Towards the Trent, the urban run-off will also deleteriously affect the water quality.

Industrial effluents have entered the Leen at various stages of its history. The area has traditionally been a centre for the textile finishing industries which employ the large scale use of chlorine for bleaching fabrics (Chapman, 1966). In 1891 there were a total of 38 bleaching and dying works along the Leen which produced a total of 4.1 m.g./d. of effluent. Only 1.1 m.g./d. was at this time disposed of via the sewerage system, the remaining 3 m.g./d. being discharged directly into the Leen. In 1968 33 bleaching and dying works were still in existence and produced 4.9 m.g./d. of effluent, all of which was discharged directly into the sewerage system (Fearn, 1969).

Another source of industrial effluent appears to be from the coal mining activities in the area. Mining effluent enters the west branch of the River Leen from Newstead Colliery, and below this point the diversity of macroinvertebrate species is

extremely poor (Saleem, 1980). High chloride levels have also been reported (Fretwell, 1976 in Saleem, 1980) resulting from either coal washing or the pumping out of a mine adjacent to the Newstead Colliery. Analysis of the S.T.W.A.* data after this incident reveals a dramatic deterioration of the water quality, and a fall in the Trent Biotic index from 7 above the effluent output to only 2 below it (Woodywiss, 1964; Saleem, 1980). Due to the activities of mining in the area, the conductivity of the water remains high for most of the time.

Thus it may be seen that the River Leen has a history of intermittent pollution. Saleem (1980) has most recently studied the pollution problems of this river and reports occasional fish mortalities having occurred as recently as 1980 when 95% of the fish, and also crayfish and swan mussels were killed in Websters pond in the west branch of the Leen (Edwards and Shepparton in Saleem, 1980). Fouling of trout ponds has also occurred (Scruby, pers. comm) due to large amounts of silt being washed downstream after cleaning operations at Newstead Abbey lakes.

The east branch of the Leen is the least affected by pollution, and just prior to the confluence with the west branch a high diversity of species is attained, the macroinvertebrate fauna including a number of pollution intolerant species of Plecoptera (Saleem, 1980). It is in this stretch of the Leen that the population study area is to be found.

1.1(ii) TOPOGRAPHY OF THE POPULATION STUDY AREA

A population of *A. pallipes* was previously known to exist in the Leen, and had been utilized periodically for research projects at Nottingham University. The location of this area, shown in

* Severn Trent Water Authority

Figure 1.1 is where the Leen flows through Top Farm at Papplewick (Map SK 43 55-53). The population study area is delimited by the footbridge and a short expanse of muddy substrate downstream, and by a large expanse upstream where the substrate is of sand and mud. The total length of the study area is 60 m, and the average width of the river at this point is 5 m.

The topography of the study area is detailed in Fig. 1.2. At this point the river is flowing over the Permian magnesian limestone which provides a firm substrate with much gravel and many large and small stones. Blocks of broken Bunter sandstone are also present from disused buildings near the river at this point, and many of these blocks were used in the construction of four wiers. These were constructed (as illustrated in Fig. 1.2 and Plates 1.1-2) to facilitate the collection of crayfish, and also to provide a way of delimiting the study area into four equivalent sub-areas for monitoring movement of the crayfish. A fifth wier was also constructed below the footbridge to enable the assessment of any downstream migrations more easily.

The habitat afforded to the crayfish is lotic, but the actual rate of flow of the river varies. After a heavy downpour of rain the level of the water rises visibly and there is an increased flow rate, but on average the depth of the river at the population study area is 20 cm. In addition, the amount of water coming downstream has been markedly affected by activities at the lakes upstream. During the period that the population was studied, Lower Lake was drained during March of 1980, and in September of the same year the level of the Upper Lake was lowered resulting in an enormous flood of water carrying great quantities of silt, debris and vegetation (Scruby, pers. comm).

1.1(iii) PHYSICAL AND CHEMICAL PARAMETERS OF THE RIVER LEEN AND POPULATION STUDY AREA

S.T.W.A. chemical analytical data are available for eight sampling points along the Leen (Fig. 1.1). These data are summarized in Table 1.1. Further analysis of the water was therefore not deemed necessary by the author, although a check was made on the conductivity above and below the confluence of the east and west branches of the river using a Digisense pH meter, model 5985-40, and a Y.S.I. model 33 S-C-T meter respectively. The sampling point at Newstead Abbey most closely represents the situation at the population study area, whilst the next point, at Papplewick moor indicates the water quality after the confluence with the polluted west branch. The following discussion relates only to the water quality of the east branch in which the population study area is located, and is based on Station 1 of the S.T.W.A. data.

TURBIDITY

In general the water was clear, the only exceptions being after heavy rainfall and during the draining of the upper and lower lakes, mentioned previously. Accordingly, the levels of suspended solids are low, ranging from a maximum of 33 mgl^{-1} recorded in March 1981, to a minimum of 1 mgl^{-1} recorded in August 1980.

OXYGEN CONCENTRATION

Disolved oxygen levels in the study area are high as might be expected for a fast flowing stream. Expressed as percentage saturation, levels of oxygen range from 86% at 25°C to 175% at 20°C. The mean oxygen level was 112% ±20%.

Expressed in terms of mgl^{-1} the range was from 8.2 mgl^{-1} to 15.9 mgl^{-1} with a mean of 12.4 ±1.8 mgl^{-1}.

pH

The mean value of pH over the two year period was 8.3 ±0.7 with maxima and minima of 9.8 and 7.4 respectively. pH was checked by the author during April of 1981 and a pH of 7.4 was obtained at the study area compared with 7.9 at Newstead Abbey (S.T.W.A.), 7.85 in the west branch, and 7.75 after the confluence of the two branches.

CONDUCTIVITY

Conductivity is a measure of the degree of ionization in the water and levels of certain ions (e.g. chloride, ammonium) are given in Table 1.1. The values for conductivity itself were low, 487 ±59 µmhos with maxima and minima of 620 µmhos and 370 µmhos respectively. This parameter was also checked by the author during April 1981 and a value of 550 µmhos was obtained for the study area, compared with 510 at Newstead Abbey (S.T.W.A.), 4,700 µmhos in the west branch, and 3,400 µmhos after the confluence of the two branches (see also 3.3).

TEMPERATURE

There was a seasonal variation of temperature which followed the same trend during both 1980 and 1981. Details of the monthly temperature variations as they relate to catch sizes are given later (Chapter 4.2). During the two year study period the range of temperatures experienced was from 0°C in January 1981 to 20°C which occurred during June and August of 1980.

HARDNESS

Hardness here is expressed as mgl^{-1} of calcium carbonate. The mean value obtained was 239 ±42 mgl^{-1} with a range of 175 to 301 mgl^{-1}.

1.1(iv) BIOLOGICAL PARAMETERS OF THE STUDY AREA

The whole of the east branch of the River Leen, including the population study area, flows through woodland, and consequently large amounts of allocthonous organic matter collects against the wiers and any other large obstructions in the water. Such material is most prevalent during the autumn and winter, and results in a eutrophic environment with great species diversity.

During 1981 samples were taken on three occasions to assess the flora and fauna of the population study area. An equivalent sample was taken on each occasion, involving a fifteen minute period of hand collection during which gravel was sifted, stones turned over, and a sample of organic debris was removed. These collections took place in April, July and October and the results are presented in table 1.2, after identification had been ellucidated from various standard identification keys. No attempt was made to assess the relative abundance of the different species, just their presence was recorded.

FLORA

The only plant life found in the population study area was attached to the rocks where anchorage against the high rate of flow was possible. *Cladophora* sp was present, and also observed was a semi terrestrial species of moss which grew on the exposed surfaces of the larger rocks and stones. No other plant life was recorded, but there was also large amounts of allocthonous organic matter from the surrounding woodlands.

FAUNA

It may be seen from Table 1.2 that the population study area is extremely rich in species, including representatives from six

different phyla. Most species were relatively abundant, the least encountered species being the leeches (Hirudinea) and water mites (*Hydracharina*). Indeed, *Hydracharina* rarely occur in fast flowing streams prefering slow moving water bodies and ponds where they are usually found attached to other insects.

The crayfish species present was confirmed to be *Austropotamobius pallipes pallipes* (Lereboullet) (Gledhill, *et. al.* 1976) and all stages of the life cycle were encountered (see Chapters 3 and 4). A discussion of the trophic relations of *A. pallipes* in relation to food availability and predation is presented at the end of this chapter.

1.2 MARKFIELD QUARRY

1.2(i) INTRODUCTION

Markfield Quarry, known locally as Hill Hole, is located in Leicestershire roughly between, and to the West of, Loughborough and Leicester. (Map SK 43 48-10). It is in the vicinity of Charnwood Forest where the geology of the area includes a group of diorites. Those near Markfield have a distinctive texture of large crystals of labradorite feldspar and Hornblende in a granophyric matrix of quartz and plagioclasse. This distinctive nature has resulted in them being known as Markfieldite (Ford, 1972). The nature of this rock type being very hard, has left it forming the highest areas in Leicestershire, with Bardon Hill at 278 m being the highest point (Rice, 1972). Close to this is the hill at Markfield, at 222 m, such that it forms a very distinctive feature of the landscape.

The first quarrying at Markfield is reported to be in 1830 (McKinley and Fagg, 1972), and in 1857 another quarry was opened at Bardon Hill by Breedon Everard. This latter quarry expanded

during the nineteenth century to become one of the countries principal sources of stone for road metalling. However, the need for each quarry to be capable of sustaining a high output to justify the expense of investment in new machinery inevitably led to the closure of some of the smaller stone workings in Leicestershire, and Hill Hole was one such quarry, closing in 1911 (Noble, pers. comm).

Flooded mineral workings are common throughout Leicestershire, and are presumably rainfilled. Certainly Markfield Quarry has no streams flowing into or out of it, and during its working life water had to be removed by steam driven buckets during the wet weather (Noble, pers. comm). During the three years that the author has visited Markfield Quarry the water level has been seen to rise by some one and a half metres due to rainfall.

Since the middle of 1980 Hill Hole has been owned by the Tarmac Roadstone Company whose intention it is to drain the quarry and refill it with waste and rubble. For part of 1980 access was not possible until permission had been granted by the new owners. However, restrictions on entry to the site imposed by the Tarmac Co. are not adhered to by the local population, and some mention of the history of the author's attempts at conducting a programme of research at the quarry are pertinent at this point!

The quarry and hill top are used locally as a recreation area, with motorbike scrambling, fishing, swimming and SCUBA training being amongst some of the activities. Thus, unlike the population study area in the River Leen, which is on private farmland and entirely closed to the public, at Markfield vandalism becomes a problem. Hence the final sampling programme achieved is a somewhat modified version of that originally envisaged.

During 1979 a weighted rope was placed along the bottom of the quarry to enable the regular sampling of a fixed transect. However, within only one week this had been removed, and bouys left to mark the presence of traps had been shot at with air rifles and sunk. It was also learned that crayfish were removed regularly by young boys using only pieces of bacon tied to fishing line. On the occasion that the author met these boys, they had collected some thirty crayfish, and claimed that they had done so every day that week. Any attempts at mark and recapture studies would thus be immediately invalidated. Later in the study, evidence that traps had been tampered with was also found, but fortunately a programme of collection, if somewhat limited, was finally achieved and is described in Chapter 2.

1.2(ii) TOPOGRAPHY OF THE POPULATION STUDY AREA

Markfield Quarry is approximately pear shaped (Fig. 1.3) and is 88 metres wide at its widest point, about 100 metres long and 50 metres deep. It is rainfilled to a depth of 8 metres, and has no streams flowing into or out of it, making it an entirely closed ecosystem. It is a still water body with only thermal currents and the activity of the wind to aid mixing of the water.

Below the water level, which is reached by a series of steep steps (Plate 1.3), the sides of the quarry fall away very steeply except in three areas as illustrated in Fig. 1.3. These areas shelve away from the vertical sides of the quarry at an angle of approximately 45° and with the exception of area 'C' which continues shelving to the bottom of the quarry, they become almost vertical after about four metres. Collections using SCUBA have been conducted across the whole of the quarry, but the major

collection site exploited was area 'A' where both snorkelling and trapping to catch crayfish were conducted. This area was chosen for several reasons, including ease of access, but it was also found during a preliminary survey using SCUBA to be the area with the highest density of crayfish. A cross section of this area is illustrated in Fig. 1.4.

The topography of the quarry consists of large and small blocks of Markfieldite on the shelving areas, and of fine mud at the bottom which is easily disturbed making navigation of a return trip across the bottom very difficult when diving. The sheer sides of the remaining parts of the quarry are relatively smooth with very few crevices to provide hides for crayfish. In addition, tree branches, old bicycles, cars, motorcycles, tin-cans, bottles and other debris of the twentieth century may be found in the quarry.

1.2(iii) PHYSICAL AND CHECMICAL PARAMETERS OF THE STUDY AREA

All the following data were collected by the author during visits to the quarry in 1981. Recordings of various parameters were made about 15 cm below the surface of the water, and again at the full extent of the cables of the recording apparatus. This may not necessarily have been the bottom of the quarry since the recorders had to be thrown out over the ledge of area 'A', but certainly it would have been at least 5 metres down if not more. In addition to the parameters (described below) which were recorded in this way, on one occasion a water sample was returned to the laboratory and chemical analysis was conducted using a La Motte Chemicals Model TRL Colorimeter and water quality analysis kit. (see Table 1.3).

TURBIDITY

In general the water was relatively clear for most of the year, except during the summer months when algal blooms tended to occur. With no currents in the water, the muddy bottom is not naturally disturbed on any great scale.

OXYGEN LEVELS

The oxygen levels in the quarry were recorded on five occasions using a YSI Model 51B Oxygen Meter. On only one occasion was the level recorded for the bottom of the quarry higher than that for the surface waters, and the range of values encountered was from 9.4 mgl^{-1} to 13 mgl^{-1} at the surface, with a mean value of 10.92 ± 1.58 mgl^{-1}, and from 8.6 mgl^{-1} to 15 mgl^{-1} at the bottom of the quarry, with a mean value of 11.09 ± 2.62 mgl^{-1}.

pH

The pH was only measured at the surface, and the range of values encountered was from 7.15 to 8.4, with a mean value of 7.85 ± 0.48 ($N = 6$). A Digisense pH meter, model 5985-40 was used.

CONDUCTIVITY

Markfieldite does not appear to leach out many ions, and conductivity readings were low. Roughly the same values were obtained for readings taken at the bottom and the surface of the quarry, and the range was from 210 µmhos to 280 µmhos with a mean of 242 ± 24 µmhos ($N = 6$). The readings were obtained using a YSI model 33 S-C-T meter.

TEMPERATURE

A seasonal variation of temperature was observed to occur. It was measured monthly at the surface for the first six months, using a mercury thermometer, and for the second half of the year

readings were taken at both the bottom and the surface using the YSI model 33 S-C-T meter. The range of temperatures experienced over the period 1980-81 was 2°C - 17°C with little difference between surface and bottom temperatures. The highest temperature recorded was after a particularly warm period in June of 1982 when both surface and bottom temperatures were recorded as being 21°C.

HARDNESS

Total hardness and its components of calcium hardness and magnesium hardness were measured on a single occasion (November, 1981) as described above. The total hardness was 116 ppm of which 70 ppm were accounted for by the calcium component, and 46 ppm by the magnesium component.

OTHER CHEMICAL DATA

See table 1.3 for the results of the analysis.

1.2(iv) BIOLOGICAL PARAMETERS OF MARKFIELD QUARRY

Evans and Block (1972) report that quarries on the edge of Charnwood Forest are steep sided, often very deep, and have a lifeless look about them. However, they do support a variety of aquatic life and perch and crayfish are reported to occur in some of the pools. This is certainly the case at Markfield Quarry but no references exist as to how the quarry initially became stocked, and it is presumed to have been from introductions by man.

No detailed collections of the flora and fauna were made at this site and the following is based upon field observations and analysis of the gut contents of several animals.

FLORA

Fontinalis antipyretica occurs in the quarry, and also in

abundance is *Cladophora* sp. which is particularly prevalent at the bottom of the quarry. A small amount of allocthonous organic matter is also found, which is presumably blown into the quarry from vegetation on the hill.

FAUNA

The crayfish species is *Austropotamobius pallipes pallipes* (Gledhill *et. al* 1976). Also present are Perch (*Perca fluviatilis*) which on the last visit to the quarry in 1982 seemed to have increased dramatically in numbers from previous visits, and pike (*Esox lucius*) (see plate 1.4).

Animal material found in the guts of crayfish from Markfield quarry, thus implicating their presence, included *Tubifex* sp., Dipteran sp. and Trichopteran sp. (Rhodes, 1980).

1.3 ON THE TROPHIC RELATIONS OF *A. PALLIPES* WITHIN THE STUDY AREAS

The following section discusses the position of *A. pallipes* in the food chain in the light of the details given about the flora and fauna present at the two population study areas, and with reference to the literature.

Despite reports that crayfish are carnivorous (Davies, 1964) or scavengers (Kossakowski, 1971; Karlsson, 1977), it is now the widely held and well established view that many species of freshwater crayfish are omnivorous and will take a wide variety of animal and vegetable matter both dead and alive (Chiddester, 1912; Roberts, 1944; Abrahamsson, 1966; Lake and Newcombe, 1975; Chapman and Lewis, 1976; Shaddick, 1976; Suter and Richardson, 1977; Thomas, 1978; Brown, 1979; Goddard, 1982). *A. pallipes* is no exception and has been shown to eat annelids (*Lumbricus* sp., *Tubifex* sp.) Molluscs (*Ammicola taylori*, *Anodonta cygnaea*, *Potamogeton crispus*), arthropods

(insect larvae and adults, *Gammarus pulex*), Nematodes (*Mermithidae*), dead fish, and a wide range of plant materials including detrital leaf litter, diatoms, bryophytes (*Fontinalis* sp.) and members of the chlorophyceae (*Cladophera* sp. *Hydrodictycn* sp.). (From personal observations and gut content analysis by Brown, 1979 and Rhodes, 1980).

Trapping of crayfish using various baits has shown that a preference for meat exists over plant matter, (Morriarty, 1972; Chapman and Lewis, 1976; Brown, 1979; Author, personal observation) and it also seems that crayfish will only eat freshly killed animal matter and will refuse stale meat (Chiddester, 1912; Davies, 1964; Morriarty, 1972). Whether crayfish are active predators however, on fast moving living animals is doubtful due to their relatively slow and clumsy movements which limit them to less active prey (Roberts, 1944; Abrahamsson, 1966).

The large chelipeds of the crayfish are less important in obtaining food than in sexual and aggressive displays (Roberts, 1944) and indeed, in large mature crayfish (> 60 mm) Abrahamsson (1966) reports a reduction of active animals in the diet. Other changes in the diet are reported, with both age variations, and seasonal variations occurring. The very small juveniles may filter feed (Budd and Lewis, 1977; Thomas, 1978), and they also utilize their own exuviae and egg capsules on the pleopods (Mason, 1970). Animal material is eaten by all age groups, but from two years and above vegetable matter is eaten increasingly. Crayfish play a large part in reducing the amount of submerged vegetation, and have even been reported grazing on land up to 0.5 m above the water's edge (Abrahamsson, 1966). This author also reports that

tanks well stocked with crayfish did not suffer from massive growths of cladophora which occurred in unstocked tanks.

The larger crayfish also tend to be cannibalistic, particularly the males, and in this way they may even help to control the population growth. Cannibalism amongst crayfish seems to be widespread throughout the many species, and juveniles and newly moulted animals appear to be the most susceptible to attack. (Chiddester, 1912; Abrahamsson, 1966; Mason, 1970; Kossakowski, 1971; Chapman and Lewis, 1976; Behrendt, 1979; Brown, 1979).

Thus it may be seen that both the River Leen and Markfield Quarry provide habitats with a rich variety of food resources available to the crayfish. In the Leen many *Anodonta cygnaeae* shells are found, which may have been preyed upon by *A. pallipes* and there is an abundance of empty shells of *Potamopyrgus jenkinsii*. Behrendt (1979) has observed crayfish pull snails out of their shells and eat them, and the author has fed crayfish on swan mussels (*Anodonta Cygnaea*) so it is likely that these could provide a food resource in the wild. In addition, the gut content analyses reported earlier show that crayfish will take insect larvae and adults, annelids, other Crustacea such as *Gammarus pulex*, nematodes and dead fish all of which occur in either one or both of the population study areas.

Whilst catching crayfish using SCUBA the author has observed *A. pallipes* eating dead fish, *Cladophora*, and also grazing up to the waters edge. Hence it is possible that terrestrial plants and animals may also form part of the diet of the crayfish. Budd and Lewis (1977) report that adult crayfish are opportunistic filter feeders, and some Markfield crayfish have been observed

to pass currents of water across their maxillipeds. To place *A. pallipes* in the food chain more exactly however, it is necessary to consider also the possible predators.

There are a large number of predators upon the freshwater crayfish, but some reported for foreign species are unlikely to be encountered by *A. pallipes*, for example salamanders, water snakes, turtles, ibis, and turkeys (Chiddester 1912). Several potential predators for the British crayfish do exist however, and these include several species of fish, mammals, and birds.

Fish species which prey on *A. pallipes* are the eel, (*Anguilla anguilla*) (Watson in Brown, 1979), the perch (*Perca fluviatilis*) (Morriarty, 1972; Mann, 1978), the brown trout, (*Salmo trutta*) (Frost and Brown 1967) rainbow trout, (*Salmo gairdnerii*) (Watson in Brown, 1979) and Canadian brook trout (*Salvelinus* sp.) (Momot, 1967; Brown, 1979). The birds include the crow (*Corvus* sp.) (Pratten in Brown 1979), and the heron (*Ardea cineria*) (Macan and Worthington 1951; Brown, 1979), whilst mammals reported to be predators are the otter (*Lutra lutra*) (Macan and Worthington, 1951) and the water vole (*Arvicola amphibius*) (Lawrence and Brown 1967). Finally the crayfish itself acts as a predator due to its cannibalistic tendancies.

In the River Leen rainbow trout have been caught upstream from the population study area, crows roost in the woods nearby, and it is unlikely that water voles exist in the area, since they prefer slow running rivers and ponds (Southern 1964). However, it is felt that the greatest danger of predation to this population lies in cannibalism, and possibly also from the bullhead minnows (*Cottus* sp.) which are likely to eat the juvenile crayfish. Bullhead

minnows were often caught in the net and appear to favour a similar habitat to the crayfish. They are reported to eat mainly invertebrates especially insect larvae, but also fish eggs and fry (Maitland, 1977) and so it seems probable that juvenile *A. pallipes* could form part of their diet.

In Markfield Quarry perch are common and also pike. Perch have been previously recorded as being predators of *A. pallipes*, but it is also possible that the pike (*Esox lucius*) will eat crayfish. Young pike tend to eat invertebrates and then fish and other vertebrates as they get older (Maitland, 1977), and in Ireland it has been reported that crayfish were present in the stomachs of nearly half the pike examined from the rivers Robe and Camlin (Morriarty, 1972). Cannibalism will also feature in the predation of the Markfield population, but another predator at this site is man (see 1.2(i)).

Thus it seems that both populations studied tend to occur at some point towards the middle of the food chain. The degree of predation upon them cannot be assessed exactly, but it would seem that the Markfield population is probably more exposed to predation pressure than that of the Leen. The behaviour of the crayfish reinforces the view that they have to be wary of predation. At Markfield Quarry a marked increase in the number of crayfish visible occurs at dusk and their nocturnal nature has also been reported in the River Darent, Kent (Ingle, 1979).

TABLE 1.1 A SUMMARY OF THE S.T.W.A. DATA FOR THE RIVER LEEN DURING THE STUDY PERIOD, 1980 - 1981

The values given are the monthly weighted mean ±1 standard deviation, except for temperature which has the mean value, and the maximum and minimum values during the study period. The numbering of the sample sites corresponds to the numbers shown in Fig. 1.1.

Sample Site	5 Day B.O.D. ($mg\,l^{-1}$)	Suspended Solids ($mg\,l^{-1}$)	Ammoniacal Nitrogen ($mg\,l^{-1}$)	Total Oxidization N_2 ($mg\,l^{-1}$)	Dissolved Oxygen (% Satn)	Dissolved Oxygen ($mg\,l^{-1}$)	pH	Conductivity (Micsm.)	Chloride ($mg\,l^{-1}$)	Alkalinity ($mg\,l^{-1}$)	Hardness ($mg\,l^{-1}$)	Temperature (°C)
Newstead Abbey (1)	3.7 ±1.8	7 ±8	0.09 ±0.09	6.8 ±1.7	112 ±26	12.4 ±1.8	8.3 ±0.7	487 ±59	36 ±13	97 ±27	239 ±42	9.1 (0-20)
Papplewick Moor (2)	2.3 ±0.9	20 ±25	0.09 ±0.06	8 ±1.3	98 ±8	11.3 ±1.2	7.9 ±0.2	2256 ±685	619 ±267	181 ±26	514 ±105	9.4 (1.5-17.5)
Bayles Mill (3)	3.8 ±2.4	16 ±10	0.26 ±0.15	8.7 ±1.6	98 ±11	11.3 ±1.0	7.8 ±0.2	2548 ±653	704 ±241	181 ±34	620 ±108	9.2 (2.0-19.5)
Bullwell Market Place (4)	3.2 ±1.4	17 ±18	0.24 ±0.17	9.1 ±1.5	102 ±11	11.6 ±0.9	7.9 ±0.2	2334 ±537	619 ±207	188 ±28	605 ±104	9.6 (2.0-19.5)
Basford (5)	3.4 ±1.8	50 ±171	0.60 ±0.31	8.3 ±2.0	109 ±16	12.0 ±1.3	7.9 ±0.2	2918 ±1018	809 ±363	235 ±31	692 ±150	11.2 (5.0-18.5)
Bobbers Mill (6)	3.5 ±2.7	26 ±63	0.41 ±0.23	8.7 ±2.1	108 ±16	12.0 ±1.2	7.9 ±0.2	2793 ±780	755 ±276	226 ±31	685 ±154	10.2 (3.5-18.5)
Hillside Lenton (7)	3.6 ±2.5	35 ±76	0.27 ±0.22	8.3 ±2.4	114 ±20	12.4 ±1.7	7.9 ±0.3	2526 ±895	680 ±313	224 ±33	657 ±138	11.2 (5.0-19.0)
Leen/Trent Confluence (8)	3.5 ±1.8	20 ±43	0.33 ±0.17	8.5 ±1.7	98 ±13	11.1 ±1.5	7.8 ±0.2	2437 ±613	616 ±222	226 ±47	630 ±140	9.9 (4.0-19.0)

TABLE 1.2 A SUMMARY OF THE FLORA AND FAUNA COLLECTED AT THE LEEN POPULATION STUDY AREA DURING 1981

ORGANISMS FOUND				APRIL	JULY	OCTOBER
INSECTA						
(a) ADULTS	(i) Hemiptera	:	*Velia caprai*	X		X
			Geridae			X
			Corixidae		X	
	(ii) Coleoptera	:	*Dysticidae*			X
(b) NYMPHYS	(i) Ephemeroptera	:	*Ephemera danica*	X	X	
			Ecdlyonurus venosus	X	X	
			Baetis rhodani	X	X	
			Centroptium luteolum		X	
			Procloen		X	
	(ii) Plecoptera	:	*Isoperla grammatica*	X	X	
(c) LARVAE	(i) Coleoptera	:	*Gyrinus spp.*	X		
			Haliplidae spp.		X	X
			Helmidae spp.	X		X
			Stratiomiidae oxycerca		X	
	(ii) Diptera	:	*Chironimidae spp.*	X	X	X
			Dixidae spp.			X
			Ceratopogonidae		X	
			Simulidae: Simulium spp.	X	X	X
			Tipulidae: Dicranota spp.		X	X
			: Tipula spp.	X	X	X
	(iii) Trichoptera	:	*Hydropsychidaea: Instabilis*	X	X	X
			Rhyacophilidae: Dobalis	X	X	X
			Philopotamidae	X		
			Polycentropidae			X

Continued.....

TABLE 1.2 A SUMMARY OF THE FLORA AND FAUNA COLLECTED AT THE LEEN POPULATION STUDY AREA DURING 1981 Cont.

ORGANISMS FOUND			APRIL	JULY	OCTOBER
		Lymnephilidae	X	X	X
		Leptoceridae		X	X
		Sericostomatinae: Goerinae	X	X	X
(d) PUPAE	(i) Diptera :	*Chironimidae*	X		
		Trichoptera	X		
CRUSTACEA	(i) Amphipoda :	*Gammanis pulex*	X	X	X
	(ii) Decapoda :	*Austropotamobius pallipes*	X	X	X
ARACHNIDA	(i) Hydracharina:	*Hygrobatidae*	X		X
ANNELIDA	(i) Oligochaetae:	*Lumbricidae: Eisenella spp.*	X		
		Lumbricidae: Lumbriculus	X	X	X
		Naididae: Nais spp.	X	X	
	(ii) Hirudinea :	*Erpobdella spp.*	X		
MOLLUSCA	(i) Gastropoda :	*Potamopyrgus jenkinsii*	X	X	X
	(ii) Lamellibranchiata :	*Sphaeridae: Pisidium*	X	X	X
		: sphaerium	X	X	X
		Unionida: Anodonta cygnaea	X	X	X
FISH		Bull headed minnow	X	X	X
		Bull headed minnow eggs	X		
ALGAE		*Cladophora spp.*	X	X	X

TABLE 1.3 A SUMMARY OF THE PHYSICAL AND CHEMICAL PARAMETERS OF MARKFIELD QUARRY DURING THE STUDY PERIOD, 1981

(a) PHYSICAL DATA. (S = surface, B = bottom)

DATE	TEMPERATURE(°C)		DISSOLVED OXYGEN(mgl^{-1})		pH	CONDUCTIVITY (μmhos)	
	S	B	S	B	S	S	B
JAN.	2	2					
FEB.	3	3					
MAR.	3	4					
APR.	6	6					
MAY	9	9					
JUN.	12	11					
JUL.	14	14	-	-	8.4	220	220
AUG.	17	16	10	9.75	8.05	240	240
SEP.	16	16	10	8.6	8.25	230	230
OCT.	12	12	13	15.0	7.15	210	230
NOV.	9	9	-	-	7.45	240	250
DEC.	5	5	12.2	12.5	7.25	280	280
JUN 82	21	21	9.4	9.6	7.80	265	260

(b) CHEMICAL DATA (P.P.M.)

Alkalinity (as $CaCO_3$) - 90.00

Carbon dioxide - 6.00

Chloride - 50.00

Free chlorine - 0.00

Combined chlorine - 0.10

Nitrates - 0.08

Nitrites - 0.04

Phosphates - 4.43

Sulphates - 45.00

FIG. 1.1

The River Leen, showing East and West Branches through to the River Trent. The urban areas (shaded) are indicated and the numbers 1-8 refer to the situation of the S.T.W.A. sampling sites. The population study area is also indicated.

FIG. 1.2

To show the topography in the vicinity of the population study area;

1. Above the ford the topography is varied and the number of crayfish found declines.
2. The substrate below the ford becomes increasingly firm with many large rocks.
3. A short muddy area with a few large stones.
4. The population study area; firm and with many rocks and stones.
5. Just below the footbridge the River becomes increasingly muddy due to silting caused by the presence of the man-made weirs.
6. Beyond the final man-made weir the substrate returns to its sandy firm nature.

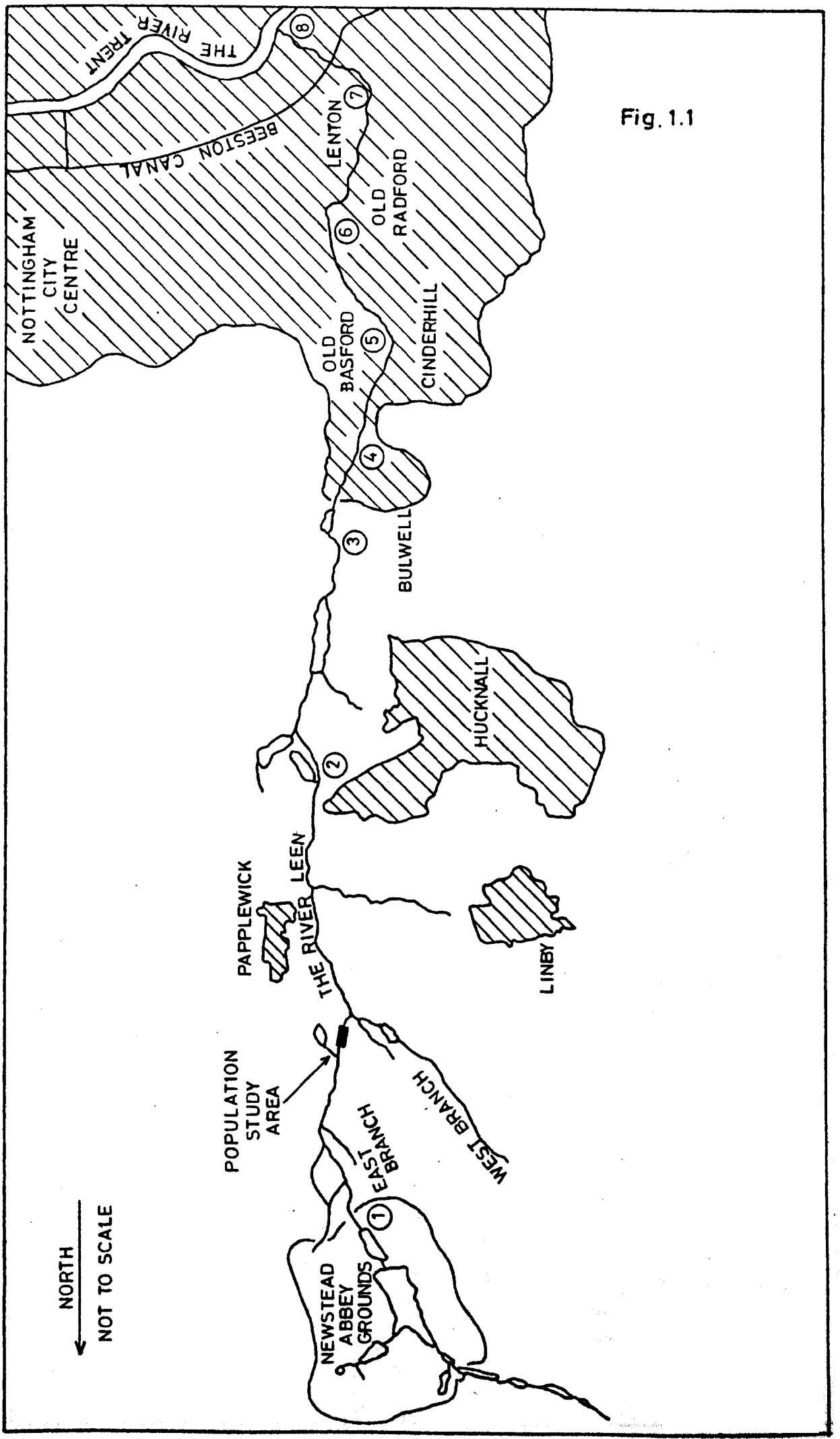

THE RIVER TRENT
BEESTON CANAL
NOTTINGHAM CITY CENTRE
LENTON
OLD RADFORD
OLD BASFORD
CINDERHILL
BULWELL
HUCKNALL
PAPPLEWICK
THE RIVER LEEN
LINBY
POPULATION STUDY AREA
EAST BRANCH
WEST BRANCH
NEWSTEAD ABBEY GROUNDS
1
2
3
4
5
6
7
8
NORTH
NOT TO SCALE

Fig. 1.1

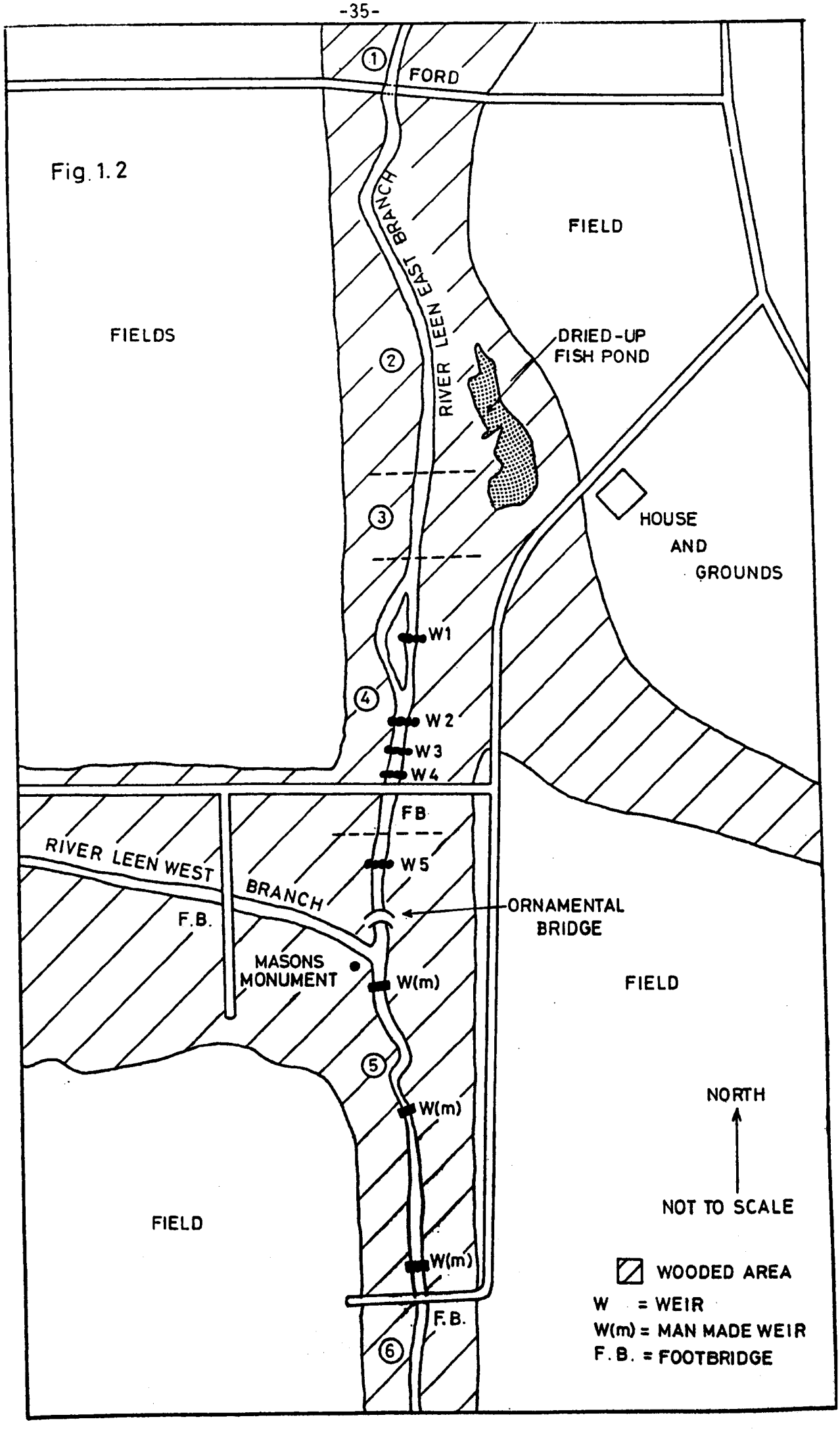


Fig. 1.2
1
FORD
FIELD
FIELDS
RIVER LEEN EAST BRANCH
2
DRIED-UP FISH POND
3
HOUSE AND GROUNDS
W1
4
W2
W3
W4
FB
RIVER LEEN WEST
BRANCH
W5
ORNAMENTAL BRIDGE
F.B.
MASONS MONUMENT
W(m)
FIELD
5
W(m)
NORTH
NOT TO SCALE
FIELD
W(m)
F.B.
6
WOODED AREA
W = WEIR
W(m) = MAN MADE WEIR
F.B. = FOOTBRIDGE

FIG. 1.3 MARKFIELD QUARRY

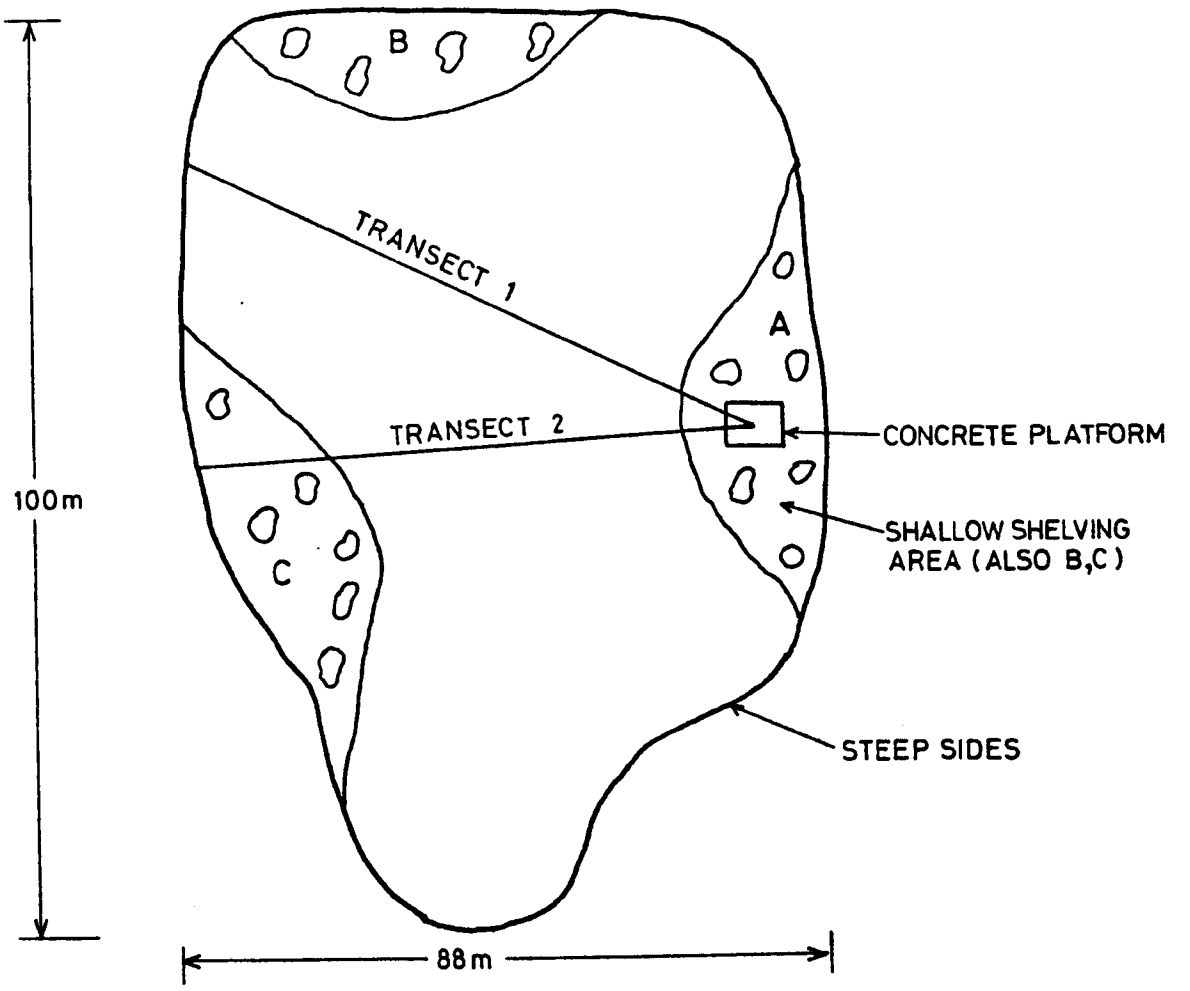
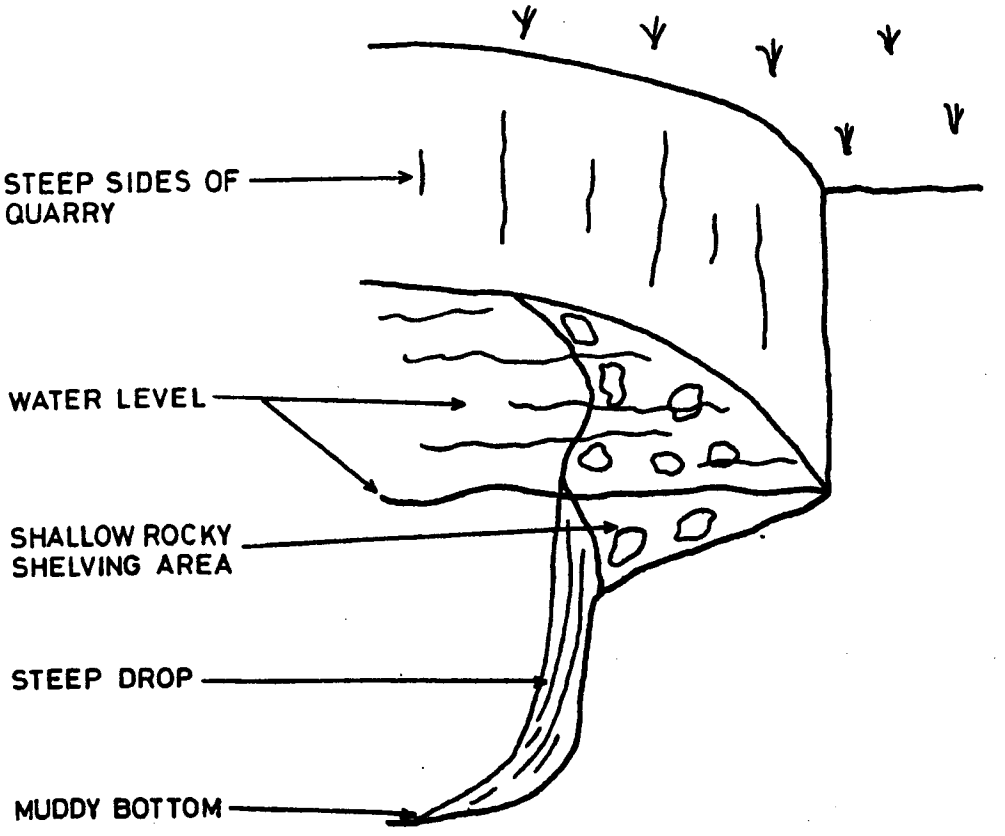


FIG. 1.4 CROSS SECTION



PLATES 1.1, 1.2

These photographs illustrate the nature of the River Leen at the population study area. The weirs shown were constructed by this author and served as a convenient method of concentrating the crayfish in order to catch them.

PLATE 1.1

PLATE 1.2

PLATE 1.3

This photograph of Markfield Quarry illustrates the steep nature of the sides. This continues even below the water line, except in Areas A-C described in Fig. 1.3.

PLATE 1.4

A pike caught in Markfield Quarry. Pike are known to be predators upon freshwater crayfish (see 1.3).

PLATE 1.3

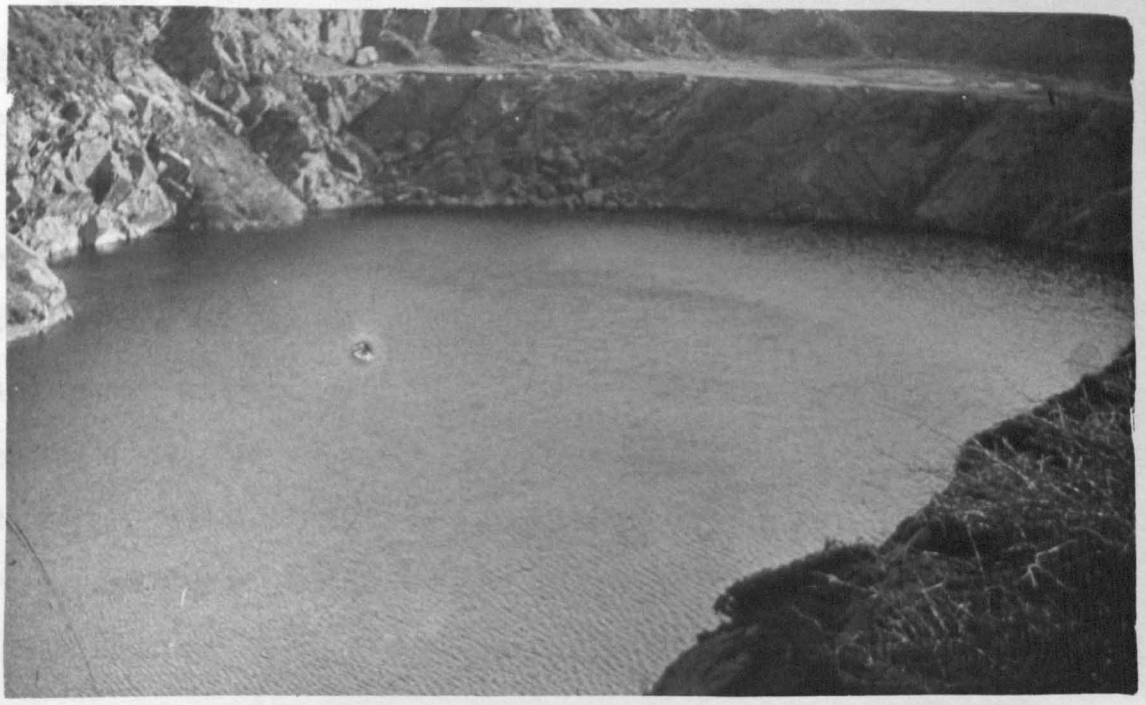
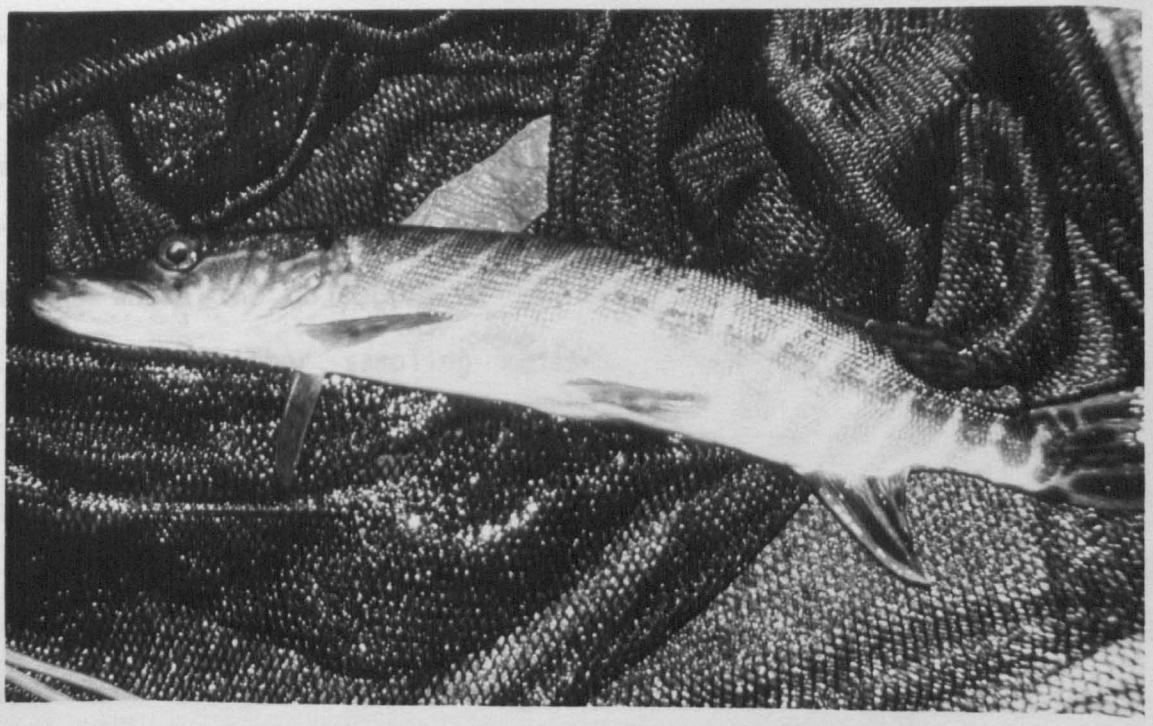


PLATE 1.4



CHAPTER 2

GENERAL MATERIALS AND METHODS EMPLOYED IN PART I OF THIS THESIS

2.1 METHODS OF COLLECTION OF ANIMALS

2.1(i) INTRODUCTION

Observation of the literature reveals that the most commonly employed methods of obtaining crayfish for research and population studies are the use of baited traps and drop nets, hand collections and hand netting, and the use of SCUBA divers. Other methods employed include dip netting (Momot and Gowing, 1977b; Huner and Rommaire, 1978), seine netting (Momot, 1967; Abrahamsson, 1971; Price and Payne, 1978), and the use of electrofishing (Abrahamsson, 1966; Hopkins, 1967; Westman *et. al.*, 1978). Each method, however, requires the water body to be of a specific nature in order to facilitate use of that method, and the only exception to this rule is trapping, which may be applied universally to all types of water bodies. Trapping, however, is not an ideal method of sampling crayfish for a variety of reasons (see 2.1(iii)) and where possible should be avoided. Brown and Brewis (1978) strongly recommend that trapping should be used only as an auxiliary sampling method where the objective of the research is a population study. Consequently, other sampling methods have been employed in this study wherever possible.

2.1(ii) THE RIVER LEEN

Monthly collections were conducted over a period of two years at the population study area (see 1.1). In addition collections were made along the whole length of the river, on one occasion, and on two occasions along all of the east branch. On each occasion the same sampling technique was employed, that of hand netting. Table 2.1 summarizes the collection periods.

Hand collection of crayfish is a commonly employed method, and takes a variety of forms. Direct hand collections without the use of a net may be conducted, and involve feeling under stones and logs for the crayfish, then quickly catching them before they escape (Hopkins, 1967; Hazlett *et. al.*, 1974; Pratten, 1980). Another example of direct hand collection is rather unique, and is that employed at an acqueduct in Northumberland which is periodically emptied enabling the simple collection of stranded crayfish (Brown and Brewis, 1978; Brown, 1979).

To catch crayfish of the 0 + year class a net is required since they are unlikely to be caught in direct hand collections (Pratten, 1980). Hand netting has been employed previously in the River Leen, (Rhodes, 1980; Rhodes and Holdich, 1982) and in the River Darent, Kent (Ingle, 1976), and also by others who have suggested that the timing of such collections should be at night (Hazlett *et. al.*, 1974; Daguerre De Hureax and Roqueplo, 1981). This is presumably due to the nocturnal foraging behaviour displayed by crayfish (Ingle, 1979).

At the population study area the river is shallow and riffles occur. Weirs have also been constructed (see 1.1) and so the site is ideal for a hand collection method, and indeed this was to be favoured over the use of traps since the former gives a less biassed sample (Abrahamsson, 1966; Pratten, 1980; see also 2.1(iii)). Hand netting was favoured over direct hand collections since it was felt that the latter would result in a high escape rate. The method involved placing a net beside, and downstream from any large stones then simultaneously lifting the stone whilst rapidly bringing the net forward. Hence nets were required to

be square so that they were flush with the substrate, and they also had metal reinforcements along the bottom edge to prevent damage to the fabric of the net. Sampling was conducted from upstream downwards in the hope that any escaping crayfish may settle at the next weir and thus be exposed to a second attempt at capture. The weirs were sampled by dismantling them from right to left, and this proved to be an effective method of obtaining a reasonable number of crayfish. During the autumn and winter months large amounts of allocthonous organic matter would collect behind the weirs, particularly at the edges of the river, and this was also sorted in the net. Juveniles particularly were found in this material.

The author conducted the monthly collections alone, and so it was most convenient to do so during the daytime. Each collection lasted for about three hours from 10.00 to 13.00 hrs, and it was felt that since the habitat was being disturbed in order to catch the crayfish from their hiding places, no great advantage would accrue from sampling at night when the animals would have been more active and possibly, therefore, more difficult to catch. The method in fact proved to be most effective and enabled a relatively unbiassed collection of all population sizes and age groups, and both sexes. Mortalities due to the method of netting were low; 0.46% in 1980 and 0.43% in 1981.

2.1(iii) MARKFIELD QUARRY

The sampling programme at the Markfield quarry site involved the use of both trapping methods, and direct hand collections with the aid of SCUBA and snorkelling. Initially a method of sampling along a transect was envisaged, by using SCUBA. This

has the advantage of regular sampling of a known area, and methods for usefully exploiting such a system have been described (Bailey *et. al .*, 1966). A similar technique was also employed in Lake Tahoe, California (Flint, 1977), where divers swam along a line and counted crayfish in a 1.5 m band either side of it. Preliminary studies of this type were conducted at Markfield Quarry, but a regular programme of research employing these methods was not feasible (see 1.2(i)).

SCUBA has been widely used in crayfish research (Abrahamsson and Goldman, 1970; Mason, 1970; Abrahamsson, 1971; Flint, 1975, 1977; Ingle, 1979). It has the advantage of being relatively unbiassed regarding the size and sex of animals caught, and allows ecological observations of the animals in their natural environments. Initial studies in Markfield Quarry involved scouting the area with the aid of SCUBA to find the general distribution of *A. pallipes* and to record the substrate. Divers swam in pairs and on one occasion a compass bearing was followed, and all animals seen within a one metre band either side of the divers were counted. Later collections simply involved snorkelling in area 'A' (Fig. 1.3) where a relatively concentrated number of crayfish were found to exist.

Sampling by snorkelling was always conducted at dusk when a pronounced increase in the numbers of crayfish was observed, as also reported by Ingle (1979) in the River Darent, where the majority of crayfish were to be seen foraging about one hour after sunset. Two people would enter the water carrying torches and net bags, and make hand collections of all the crayfish seen. This method is less biassed than trapping, but must also involve

some element of bias. In the presence of a predator the behaviour of crayfish is highly modified (Stein and Magnusson, 1976). They select substrates which afford them the most protection, and feeding, walking and drinking activities are suppressed, whilst defensive actions such as burrowing and chelae displays are increased. Such predator response displays (see Hayes, 1977) were often encountered in Markfield Quarry when the torch light fell on the crayfish. This had the advantage of making these individuals relatively easy to catch, but must also be where the element of bias in catchability is to be found. The modification of the predator response varies according to the size of the animal, and escape patterns are more commonly exhibited by smaller animals and females, whilst the larger males are more inclined to employ an aggressive posture (Stein and Magnusson, 1976). Indeed, it was observed in the Quarry that the smaller animals tended to employ the tail flip escape response the most readily, and having evaded initial capture would invariably hide under blocks of Markfieldite, thus becoming virtually impossible to catch. The sex ratios of samples caught whilst snorkelling were always approximately 1:1 but the smallest animal caught had a carapace length of 15 mm, compared to 5 mm for newly hatched juveniles.

Thus, to obtain data relating to the population structure and sex ratios, snorkelling was employed, but trapping on a monthly basis was carried out to monitor factors such as moulting and breeding condition. The traps consisted of black plastic drainpiping, 15 cm in diameter, and 45 cm long, with a funnel entrance at one end (the hole was about 2.5 cm diameter), and removable wire

mesh at the other, for emptying the traps. Ten traps were employed in two lines of five, attached to nylon cord. They were baited with ox-liver and placed in area 'A' (see 1.3). The traps were set at dusk and collected the first thing the following morning. This served the dual purpose of reducing the chance of vandalism, and increasing the possible catch. It has been shown that a correlation exists between catch size and the time interval between emptying and setting the traps (Brown, 1979). The chance of escapes occurring increases in proportion to the length of time that the traps remain unemptied. In some countries it is reported that traps are set and lifted on the same night to ensure optimal catches (Lindqvist, in Brown, 1979). The timing of the visits to Markfield Quarry are shown in Table 2.1.

Trapping as a method of catching crayfish is the most commonly employed technique (Woodward, 1877; Camougis and Hichar, 1959; Abrahamsson, 1966, 1971; Momot, 1967; Abrahamsson and Goldman, 1970; Mason, 1970; Morriarty, 1971; Flint, 1975, 1977; Morrissy, 1975; Brown and Brewis, 1978; Huner and Romaire, 1978; Brown, 1979; Rhodes and Holdich, 1979; Pratten, 1980; Daguerre De Hureax and Roqueplo, 1981). However, its limitations must be fully appreciated if it is to be used effectively, particularly when used as part of a population study rather than simply as a means of collection. Examination of both size and sex ratios of animals caught in traps in fact produce biassed results, and are not indicative of the true nature of the population.

Catchability amongst crayfish populations is affected by several factors, and not all animals will be of equal catchability (Morrissy, 1975). The habitat preferences of certain animals

may not coincide with trap placements, and the activity of the crayfish will also affect their vulnerability to capture. A positive correlation has been shown to exist between catchability and seasonal water temperature (Morrissy, 1975), and this is undoubtably one of the factors affecting activity. The catchability of females is also variable. Their behaviour in response to traps alters throughout the life cycle, and Brown (1979) reports for *A. pallipes* that the only period when the sex ratio of trapped samples approached unity was when hatching had occurred, but before fertilization. The tendancy is for trapped samples to contain more males than females, and in addition, it appears that since dominance order decides trap entry (Brown, 1979), there are more adults caught than juveniles.

Finally, there is some evidence that a learned response to traps is exhibited by crayfish. For marron *Cherax* in Australia it is reported that previous capture would affect the subsequent catchability of some animals, making them less likely to be caught (Morrissy, 1975). For *A. pallipes* it is reported that some males may even become 'trap-happy', whilst females remain 'trap-shy' to multiple recaptures (Brown, 1979). Learning would not be a problem regarding the Markfield population since all animals were removed to holding tanks at the University of Nottingham (see Part II) and maintained for experimental use. Mark and recapture studies were not conducted since the size of the quarry prohibited a large scale collection of animals, and also since continual removal of crayfish was occurring by other visitors to the quarry.

2.1(iv) NANPANTAN RESERVOIR

Nanpantan reservoir was not one of the population study sites, but was known to contain a large population of *A. pallipes* (Jay and Holdich, 1981). It is situated just outside Loughborough (map reference, SK 43 51-17-), and it forms an intermediate type of habitat between the River Leen and Markfield Quarry, in that it is a large reservoir, but is not an entirely closed habitat, being fed and drained by Wood Brook. In November, 1979 the reservoir was drained to facilitate cleaning, and so collection of crayfish was easily achieved by walking across the bottom mud and simply picking up the stranded animals. Several hundred animals were collected in this way and were measured for comparison with the study populations. They were then held for experimental purposes.

2.2 MARKING TECHNIQUES AND THE RELEASE OF MARKED ANIMALS

Animals collected from each site were returned to the laboratory for measuring and for individual marking. Those from Markfield Quarry were maintained in concrete holding tanks for experimental use, whilst those from the River Leen were marked and returned the same day. Crayfish caught from each weir were maintained in separate containers and returned to the weir from which they were caught. Release involved actually placing the animals under stones and holding them there until settled. This ensured that each crayfish was returned to almost exactly the same position as prior to its capture, and thus true movements would be indicated, if, on subsequent recapture, an animal was found to be at a different site. Simply releasing the animals a short way upstream in the hope that they would be carried back into the weir proved

ineffective. In the majority of cases, animals released in this way would, on finding the bottom, walk upstream until they located a suitable hide.

The reasons for marking the crayfish were several. Individual marks enabled the monitoring of movement, and also enabled growth studies to be performed. In addition, it was possible to estimate the size of the population from a programme of mark and recaptures. The individual marking system was also employed for this as it was felt unnecessary to develop an alternative marking system for the population studies.

Thus it may be seen that it was necessary for the marking technique to satisfy various criteria in order to accomplish all that was required of it. For the estimation of population size it is necessary that the mark does not affect survival, either directly or due to predation, nor must it affect subsequent catchability, for example, by making the animal more conspicuous, or by resulting in abnormal behaviour which may either make it easier or more difficult to catch. In addition, the mark must persist for sufficiently long for it to be observed on subsequent recapture. Regarding growth, any system of marking employed must be specific to individual animals, must not be lost at the moult, and must not hinder or prevent ecdysis. Individual marking is also necessary for monitoring movement, and common to all concerns, is the requirement that the marks should not be liable to any confusion with natural marks or mutilations.

There are several methods of marking invertebrates (see Southwood, 1977, for review). Mutilation is a common technique with crustaceans, and may involve pleural clipping, the punching

of holes into areas such as the telson, or limb removal (Svardsson, 1949; Slack, 1955; George, 1957; Simpson, 1961; Wilder, 1963; Momot, 1967; Hazlett *et. al.*, 1974). However, this method has the problem that it may affect growth, or that the marks may be lost after moulting. Confusion with natural marks may also arise. Injection of dyes into the centre of the abdomen has also been employed with Crustacea (e.g. Menzel, 1955; Slack, 1955; Penn, 1975), but this does not allow individual recognition, and high mortalities may occur.

Individual marking may be achieved with the use of dyes on the exoskeleton (e.g. Camougis and Hichar, 1959), but this of course is lost at ecdysis. Tagging is more acceptable, and may also be individual, but again may be lost at moulting. Increased mortalities may also arise and growth may be affected. It has, however, been a widely employed method of marking (e.g. Loosanoff, 1953; Simpson, 1961, 1963; Wilder, 1963; George, 1965, Penn, 1975). Certain tags have been devised which are not lost at the moult, but the moult increment at ecdysis is affected (see Penn, 1975), and so they are unsuitable for studies of growth. Tagging for the monitoring of movements has sometimes involved the use of radioactive sources (e.g. Merkle, 1969), and radio transmitters or ultrasonic tags have also been employed (e.g. Bottoms and Marlow, 1979). However, the latter have the disadvantage that the animal itself must be reasonably large to accommodate the tag. This author conducted a trial run using a radio transmitting source. It was conducted in an artificial river at the University, and only the very largest crayfish were suitable. The tag resulted in behaviour modifications since it restricted hide entry, and

mortalities also resulted. Usually such tags have been employed on larger crustaceans such as large crabs.

Thus it may be seen that certain disadvantages occur with all of the above methods. However, the method which has been generally accepted, and is now so common as to warrant being called the standard marking technique for crayfish, is that of Abrahamsson (1965). He employed a method of cauterization of the cephalothorax using a soldering iron, which had also been used successfully with lobsters (Dybern, 1965). This destroys the underlying pigment cells and also the pigment itself, leaving a red mark on the carapace. By employing a specific pattern of marks, individual numbering may be achieved. That employed by this author is slightly modified from Abrahamsson (1965) and is shown in Fig. 2.1. Several thousand crayfish may be individually marked in this manner and a total of 1,271 animals from the Leen were thus marked. Only animals above 10 mm carapace length were marked, and a soldering iron filed to a point was employed. Marked and recaptured animals had their marks reinforced if they had moulted.

Several authors have employed this technique (e.g.Abrahamsson, 1965; Morriarty, 1972; Flint, 1975; Brewis, 1978; Price and Payne, 1978; Brown, 1979; Pratten, 1980; Rhodes, 1980). It has the advantage of persisting over several moults, the maximum observed being four (Brown, 1979). It does not affect growth or survival, and the marks are easily recognized. Catchability however, is unnaffected since in this study the collection technique does not first involve seeing the crayfish. The only disadvantage could be that the red spots would lead to increased predation, but since the animals

are largely nocturnal (Ingle, 1979) it is doubtful whether this has much effect.

2.3 OBSERVATIONS ON THE GENERAL CONDITION OF ANIMALS

All animals caught from each site had various aspects of their general condition recorded. This was in order to facilitate the timing of events in the life history and to assess the state of health of the crayfish. Also recorded was the date of sampling and, for the River Leen population, the reference number accorded to each individual animal. The weir they were caught at was also recorded. Thus, in addition to the timing of the life history, the movements and growth of individual crayfish could also be assessed, and related to a timescale.

(i) SEX

Above 10 mm carapace length it is a simple matter to distinguish between the sexes. The first two pairs of pleopods on the abdomen of male crayfish are modified into forward pointing extensions for the passing of spermatophores (Huxley, 1896; Thomas, 1976). Below 10 mm no attempts to sex the crayfish were made, and they were simply recorded as juveniles.

The presence of spermatophores was also recorded, and was taken to indicate that fertilization had occurred (Ingle and Thomas, 1974). Females in the ovigerous state had their eggs counted, which are attached to the pleopods under the abdomen, and after hatching the presence of any juveniles clinging to the pleopods was also recorded. Juveniles were designated as being in the 0+ year class for the first summer, and 1+, 2+, 3+, etc. for the second, third, fourth, etc. summers, moving up one year class each July when the new juveniles hatched.

(ii) MOULT CYCLE

Ross Stevenson (1974) has described the moult cycle of *Orconectes* sp. in some detail, and states that the description probably applies to all crayfish. In all, thirteen stages are described, but only four are recognized in this study (excluding moulting itself), and this was felt to be sufficiently detailed. These stages are; firstly, 'about to moult', equivalent to stage D_3 of the Ross Stevenson classification, where the separation of the epidermis from the cuticle may be observed at the edge of the uropods and telson. At this stage, the entire body reaches a point at which enough of the old cuticle has been reabsorbed that it may be compressed easily between the fingers. Moulting follows, and then the next stage recognized by this author was recorded as, 'just moulted'. It is equivalent to A_1 and A_2 (Ross Stevenson, 1974), and is when the carapace is still very soft and feels quite slippery. 'Newly moulted' was recorded as the next stage, (equivalent to C_1 - C_3, Ross Stevenson, 1974) and was taken to indicate the fact that the crayfish had obviously moulted recently, probably within two weeks of the observation. At this stage the crayfish appear very clean, being free of all epizooitic growths which occur on the integument of unmoulted animals. Finally, there is the 'intermoult period', (C_4, Ross Stevenson, 1974). The length of this stage is variable, and was in fact given two designations; the overwintering intermoult period, which occurs outside the growth period and lasts several months, and the summer intermoult period, when there is a possibility that another moult cycle may be completed.

(iii) DAMAGE

All damage and injuries to the exoskeleton and limbs of the crayfish were recorded, and any fishing mortalities due to the method of catching the crayfish were noted. Chelae loss and regeneration amongst crayfish is common and may indicate social interactions amongst the individuals of a population. In addition, damage to areas such as the telson or rostrum were also recorded. In some cases rostrum damage is reported to be a common occurrence (e.g. Hopkins, 1967), thus complicating the measurement of the carapace length. With the Midlands populations this was not a problem, and when rostrum damage did occur it was obvious due to the distinctive nature of this feature in *A. pallipes* (Gordon, 1963; Thomas, 1974).

(iv) DISEASE

Austropotamobius pallipes is affected by the endoparasite *Thelohania conejeani* Henneguy (Cossins, 1972). It is a microsporidian parasite which attacks the muscle tissue of the living crayfish, and leads to deterioration of muscle function and death. Also known as porcelain disease, it gets its name from the fact that when it is in an advanced state the muscle tissue is white and opaque when viewed through the transparent sternum (see Plate 2.1). Normal animals have a translucent abdominal muscle. Thus it is only animals in an advanced state of infection which may easily be recognized as diseased. All animals exhibiting this condition were recorded.

2.4 THE MEASUREMENT OF ANIMALS

All the animals caught each month were subjected to a series of measurements (see Fig. 2.2). Measurements were made accurately

using Vernier calipers to 0.1 mm. The measurements were as follows:

(i) CARAPACE LENGTH, from the tip of the rostrum to the postero-medial edge of the carapace. If the rostrum was damaged, this measurement was not taken.

(ii) CARAPACE WIDTH, at its widest point.

(iii) TOTAL LENGTH, from the tip of the rostrum to the posterior rim of the telson (excluding the setae).

(iv) CHELA LENGTH, from the carpal joint to the tip of the propodus.

(v) CHELA WIDTH, at its widest point.

(vi) CHELA DIGIT, the length of the moveable digit.

(vii) ABDOMEN WIDTH, at the second, and widest segment.

(viii) TELSON LENGTH, from its articulation with the sixth abdominal segment to its posterior rim, excluding setae.

(ix) TELSON WIDTH, at its widest point.

(x) ROSTRUM LENGTH, from the tip of the rostral spine to the anterior end of the post-orbital ridge.

(xi) ROSTRUM WIDTH, between the left and right post-orbital ridges.

(xii) WEIGHT. The crayfish were held rostrum down to empty any water in the gill chambers, and they were then dried with a towel to remove any surface water. They were weighed accurately to 0.01 g. Juvenile crayfish of the 0+ year class were weighed to 0.0001 g. The previous measurements were made using a binocular microscope and calibrated graticule. Where a large number of 0+ juveniles were caught, only a randomly selected proportion were measured.

The accuracy of the measurements was determined by comparing the original measurements of marked animals with the measurements taken on subsequent recapture, providing they had not moulted in the intervening period. The mean error was 0.21 mm for the carapace length, with 99% confidence limits about the mean, of ±0.55 mm (2½ S.D., N = 68). The majority of measurements were in fact well within the confidence limits, and were often accurate to within ±0.1 mm.

The carapace length was taken as the reference dimension in all growth and morphometric studies since it was felt to be the most accurate measurement taken, and was not subject to variations which may be introduced due to abdominal flexibility, should the total length have been chosen.

2.5 THE STORAGE AND ANALYSIS OF FIELD DATA

Specific analytical and statistical techniques which were used are given at the relevant points in the text. All the data was transformed into a numerical code system, suitable for storage on the Nottingham ICL 2900 computer. Analysis was facilitated by use of the Statistical Package for the Social Sciences (SPSS, for ICL 2900, Version 4.2 (IBM Release 8.1), 1981).

TABLE 2.1 A SUMMARY OF THE COLLECTIONS MADE AT EACH SITE

RIVER LEEN 1979 - 1980			RIVER LEEN 1981		
Date	Description of Collection		Date	Description of Collection	
5/12/79	To establish the		5/1/81	JANUARY, monthly collection	
10/12/79	collecting areas and		7/1/81	JANUARY, RECAPTURE 1	P*
18/12/79	build weirs.		9/1/81	JANUARY, RECAPTURE 2	P*
8/1/80	JANUARY, monthly collection		2/2/81	FEBRUARY, monthly collection	
7/2/80	FEBRUARY, monthly collection		2/3/81	MARCH, monthly collection	
7/3/80	MARCH, monthly collection		6/4/81	APRIL, monthly collection	
2/4/80	APRIL, monthly collection		8/4/81	APRIL, RECAPTURE 1	P*
5/5/80	MAY, monthly collection		10/4/81	APRIL, RECPATURE 2	P*
2/6/80	JUNE, monthly collection		4/5/81	MAY, monthly capture	
7/7/80	JULY, monthly collection		8/6/81	JUNE, monthly capture	
9/7/80	JULY, RECAPTURE 1	P*	6/7/81	JULY, monthly capture	
11/7/80	JULY, RECAPTURE 2	P*	8/7/81	JULY, RECAPTURE 1	P*
4/8/80	AUGUST, monthly collection		10/7/81	JULY, RECPATURE 2	P*

Continued.....

TABLE 2.1 A SUMMARY OF THE COLLECTIONS MADE AT EACH SITE continued

RIVER LEEN 1979 - 1980			RIVER LEEN 1981		
Date	Description of Collection		Date	Description of Collection	
18-22/8/80	Survey of whole of Leen		3/8/81	AUGUST, monthly capture	
3/9/80	SEPTEMBER, monthly collection		5/8/81	AUGUST, WHOLE RIVER	p*
8/9/80	SEPTEMBER WHOLE RIVER	p*	7/8/81	AUGUST, WHOLE RIVER RECAPTURE	p*
10/9/80	SEPTEMBER WHOLE RIVER RECAPTURE	p*	27/8/81	SEPTEMBER, monthly capture	
1/10/80	OCTOBER, monthly collection		28/9/81	OCTOBER, monthly capture	
3/11/80	NOVEMBER, monthly collection		30/9/81	OCTOBER, RECAPTURE 1	p*
1/12/80	DECEMBER, monthly collection		2/10/81	OCTOBER, RECAPTURE 2	p*
			2/11/81	NOVEMBER, monthly capture	
			30/11/81	DECEMBER, monthly capture	

P* POPULATION ESTIMATE MADE.

TABLE 2.1 A SUMMARY OF THE COLLECTIONS MADE AT EACH SITE continued

MARKFIELD QUARRY 1979 - 1980		MARKFIELD QUARRY 1981 - 1982	
Date	Description of Collection	Date	Description of Collection
30/10/79	DUSK DIVE, feasibility survey	13/1/81	JANUARY, monthly trapping
6/11/79	DAY TIME DIVE, Transect survey	4/2/81	FEBRUARY, monthly trapping
5/9/80	Snorkell collection	4/3/81	MARCH, monthly trapping
5/11/80	NOVEMBER, monthly trapping	6/4/81	APRIL, monthly trapping
5/12/80	DECEMBER, monthly trapping	6/5/81	MAY, monthly trapping
		11/6/81	JUNE, monthly trapping
		22/6/81	Snorkell collection
		3/7/81	JULY, monthly trapping
		1/8/81	AUGUST, monthly trapping
		26/8/81	SEPTEMBER, monthly trapping
		5/10/81	OCTOBER, monthly trapping
		20/10/81	Snorkell collection
		3/11/81	NOVEMBER, monthly collection
		1/12/81	DECEMBER, monthly collection
		6/82	Snorkell collection

FIG. 2.1 TO SHOW THE PATTERN OF MARKING ADOPTED

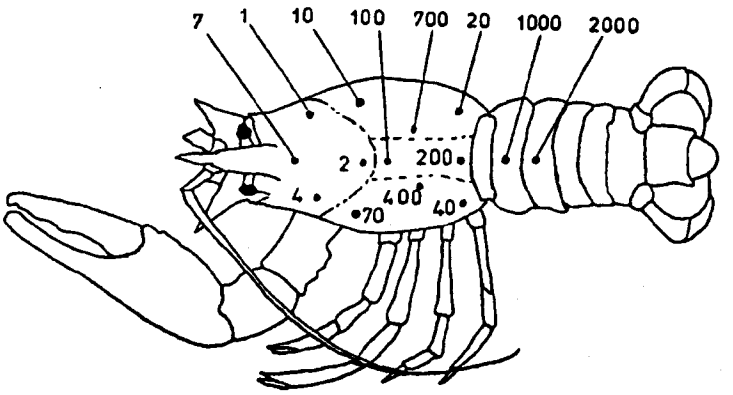
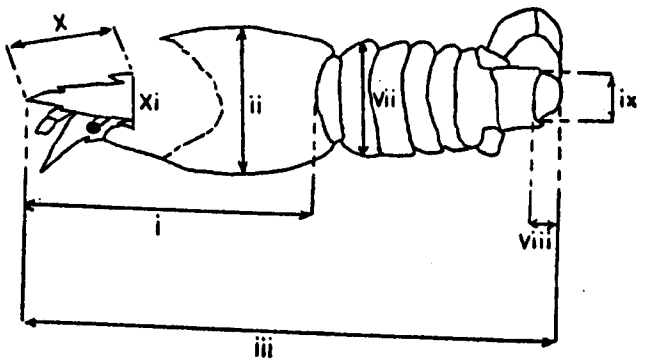


FIG. 2.2 TO SHOW THE MEASUREMENTS TAKEN
(NUMBERS i-xi REFER TO THOSE IN THE TEXT)



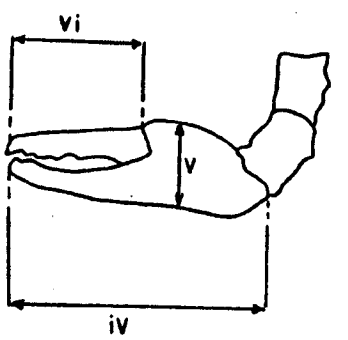
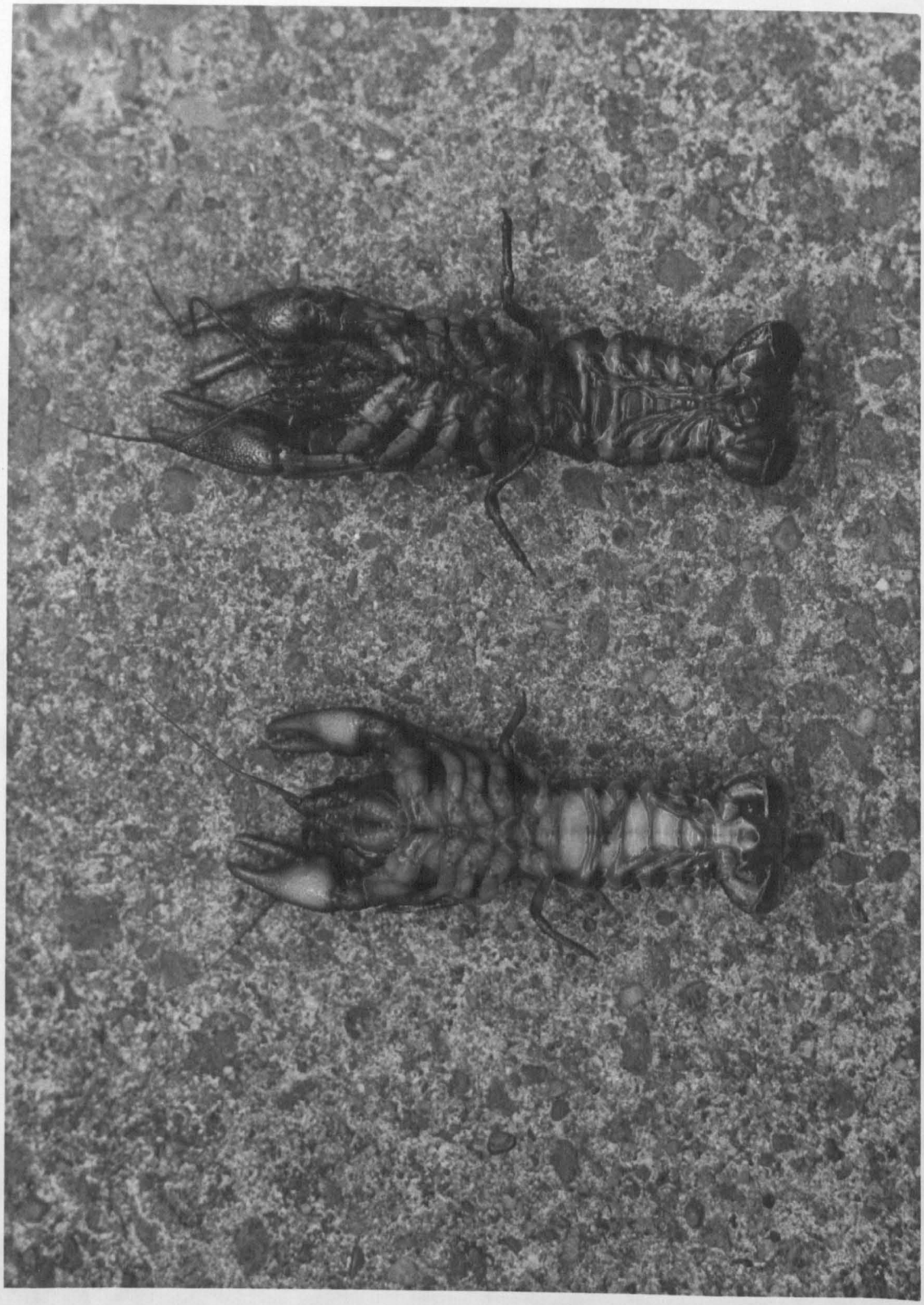


PLATE 2.1

This photograph illustrates the appearance of crayfish diseased with the parasite *Thelohania contejeanii* as compared with a normal crayfish. The abdomen of the diseased animal appears white viewed through the transparent sternum, whilst normal animals have a translucent abdominal muscle, which appears dark in the photograph.

PLATE 2.1



CHAPTER 3

THE BIOLOGY OF *A. PALLIPES* IN THE MIDLANDS

3.1 INTRODUCTION

The requirement for the build up of knowledge of the biology of *A. pallipes* from different sites in Britain has been discussed (see Introduction to Section I). This has recently become an urgent requirement so that what will effectively constitute a 'baseline-survey' of *A. pallipes* in Britain is available for future reference if, and when, the non-indigenous species *P. leniusculus* is able to establish itself in our rivers and other waterbodies. Only with this knowledge will it be possible to establish exactly what effect these introductions may have had.

From an ecological point of view it is bad practice to proceed with the introduction of any alien species without first having a thorough knowledge of the ecology of both that species, and the habitat into which it will be introduced. This point has been well illustrated in East Africa. *Procambarus clarkii* were introduced into Lake Naivasha, Kenya, in 1971, and they have now become well established. They have seriously affected the gill net fisheries by upsetting the nest and brooding areas of Tilapia, and also by damaging fish caught in the nets, making them unmarketable. Furthermore, the crayfish themselves are on the whole unmarketable since they have developed wasted tail muscles. Hence it has too late been recommended that *P. clarkii* should not be introduced into areas supporting gill net fisheries (Lowery and Mendes, 1977).

In Britain, except in Scotland, *P. leniusculus* (the species generally being introduced - see Introduction), would not occupy an empty niche, but would be competing directly with *A. pallipes*. Hence the effects to the overall ecology of the waterways may not be particularly devastating, except towards *A. pallipes* itself! There is, however, the danger of introducing the crayfish plague (see Introduction), and that this could spread faster than its host, *P. leniusculus*. This could have rather more far reaching effects, which could possibly be of commercial and recreational concern. Should the plague erradicate *A. pallipes*, then in the absence of this benthic invertebrate algal blooms may occur (Abrahamsson, 1966; Spitzy, 1972), and if weed choked rivers resulted, both fishing, and sailing and boating, could seriously be affected.

Thus, in this chapter on the biology of *A. pallipes* several factors are discussed. Towards the idea of a 'baseline-survey', additional information is made available concerning the ecology of *A. pallipes*, in this case from the two Midlands populations previously described (see Chapter 1). The life cycle is examined and factors that may affect the local distribution of crayfish are discussed. In addition to forming a base-line study, information may be gained to help assess whether *A. pallipes* could form an alternative food source to *P. leniusculus*, and thus remove the need for its importation, and its associated ecological problems. Details of the life cycle such as the timing of the release of eggs and juveniles are of relevance in this connection. Such information would help in the formulation of guidelines as to when the cropping of populations of *A. pallipes* is either viable,

or an ecologically sound proposition.

When considering both base-line studies, and the question of exploiting stocks of *A. pallipes*, population studies are also involved, in terms of population size (4.1), and population structure (4.2), and are discussed in chapter 4.

3.2 ASPECTS OF THE LIFE HISTORY OF *A. PALLIPES*

3.2(i) A DESCRIPTION OF THE LIFE HISTORY

In brief the life history of *A. pallipes* involves a yearly cycle of events, thus; a dormant over-wintering period in which no growth occurs, but development of eggs proceeds, is followed by a period of growth in the summer, when moulting occurs. At the beginning of the summer any eggs will have fully developed, and will hatch. Towards the latter part of the autumn fertilization and egg laying occur, moulting ceases, and it is once again the dormant over-wintering period. Such a brief description, however, does not allow for variability in any of these events. For instance, the moult frequency varies with age, and the timing of events, such as the onset of sexual maturity, are not discussed. A more detailed description of the life history then, will describe how these basic events change throughout the entire life of the animal. Fig. 3.1 is a summary of a generalized life history for *A. pallipes*. It traces their development from the beginning, as eggs on the maternal pleopods, through four year classes, to the 3+ year class and the onset of sexual maturity.

Table 3.1 summarizes the timing of events in the life histories of the two Midlands populations, and compares them with other populations of *A. pallipes* in Britain. During the winter when temperatures are low (see 1.1 and 1.2) the activity and foraging

behaviour of crayfish is reduced (Brewis, 1978). This is the dormant period when no growth occurs until the temperature begins to increase. In decapod crustaceans growth is achieved by moulting. It was first observed to occur in the River Leen population and then slightly later at Markfield Quarry. In 1980 and 1981 newly moulted animals were first observed in the Leen on the 2nd, and 8th of June respectively. Hence moulting had occurred at the end of May, and the beginning of June. In Markfield Quarry, however, no moulted animals were caught until the 22nd of June, 1981, indicating that it had started slightly later than in the Leen. By contrast, in 1982 an additional collection was made in Markfield Quarry early in June (7.6.82). Newly moulted animals were present in the sample, and this was related to the fact that a particularly warm period at the end of May had raised the water temperature to 21°C, and had resulted in earlier moulting in that year.

Growth and the timing of moulting are temperature dependent (see 5.1). Thus moulting is restricted to the summer months when temperatures are in excess of about 10°C, and it ceases towards the winter as temperatures fall again. At Markfield Quarry the last newly moulted animals were observed in October, which compares with October (1980) and November (1981) in the River Leen. Hence the length of the growing season at the former study site is shorter than that of the latter, where a single juvenile individual was even observed to have moulted as late as December! The lengths of the growing seasons, with upper and lower confidence limits, are obtained from the interval between visits when moulting was first and last observed, and plus or minus a period of seven days. This latter figure is estimated as the time taken to develop

from the moult-stage to the newly moulted stage (see 2.3, derived from Ross-Stevenson, 1974). The lengths of the moulting seasons are; 112 - 126 days at Markfield Quarry (1981), and 113 - 127 days (1980), and 140 - 154 days (1981), in the River Leen.

The first moult observed at each site appears to occur synchronously for a large number of animals, but no evidence was found to suggest that subsequent moults occurred synchronously. The above data relates to the timing observed for moulting of the whole population. By treating the population as sub-populations of mature, and immature males, and females, however, it is possible to get a better idea of the real situation. Maturity for both sexes is taken to be 25 mm (C.L.) (see below). Table 3.2 shows the proportion of animals in each sub-population which have completed at least one moult, and at different times of the year.

It will be seen that although moulting occurs throughout the entire growing season, not all of the animals moult for the entire period. Simply comparing the sexes at the start of the growing season, in the River Leen it will be seen that more males caught in June had moulted than females (e.g. 1980 data, 65.9% of males, and 37.3% of females. The situation observed for Markfield Quarry is the reverse, but since trapping introduces bias (see 2.1) it is felt that the true situation is probably similar to the Leen). Breaking the sub-populations down further, it is apparent that of the males from the River Leen which have moulted, in June, for example, the vast majority are immature crayfish, and only a small proportion of mature animals have done so (95% of immature males have moulted, and only 38.1% of mature males in 1980). The situation is similar for mature and immature females,

and for the Markfield Quarry population (see Table 3.2).

In conclusion, adult males moult earlier than adult females. All males have completed at least one moult by September, whilst this is not the case for females until November. For both sexes immature animals moult the earliest, and all have completed one moult by July. Few adults moult at the beginning of the season. These points are well illustrated in Figs. 3.2 a, and b. Fig. 3.2a represents crayfish caught in June 1980 in the River Leen, and illustrates for both sexes that it is the largest animals which have not yet moulted. Fig. 3.2b represents the situation in November 1980, at the end of the growing season. It will be seen that the animals continuing to moult are immature, indicating that not only do they begin moulting prior to the mature animals, but that also they cease moulting later. Hence they enjoy a considerably longer growing season (see also 5.1).

The moult frequency of immature animals is greater than that of mature animals due to the longer growing season which they experience. A detailed description of moult frequency occurs in 5.1. Briefly however, immature crayfish undergo several moults per year and the frequency decreases with age. Ovigerous females moult only once per annum, after the eggs have hatched, whilst larger males may, or may not moult more than once. All animals moult at least once per year.

Sexual maturity is achieved for both sexes at about 25 mm carapace length. In the River Leen this size may be attained by October for some individuals of the 2+ year class, but it is not attained until the 3+ year class in Markfield Quarry (see 5.1). The smallest female observed to be carrying eggs was a

specimen from the River Leen with a carapace length of 23.3 mm. However, the majority of animals do not become sexually active until they are larger, and therefore also older, and in the 3+ age class and above. In the Leen population 30 mm (C.L.) is the size of the over-wintering 3+ age class. Only 34.6% and 21.6% of the total ovigerous females caught during 1980 and 1981 were below this size. 30 mm (C.L.) represents the 4+ age class at Markfield Quarry (see tables 5.6 and 5.7) and no animals were caught less than this size which were berried. The proportion for the stock caught in November 1979 at Nanpantan was 17.2%.

Mating of mature specimens of *A. pallipes* has been described by Ingle and Thomas (1974). About two weeks prior to egg laying, the male passes a packet of sperm, known as the spermatophore, onto the sternum of the female. Spermatophores were first observed in Markfield Quarry in October, but no ovigerous females were caught until December, and so it was not possible to put a precise time limit to the interval between fertilization and egg laying. Similarly in the Leen, where the first observations of spermatophores occurred in November, at the same time as the first eggs. However, based on these observations it is reasonable to assume that in both populations fertilization will have occurred in mid-October, with egg laying occurring early in November.

The eggs are laid into mucus secreted from the glair glands. This helps to dissolve the spermatophore and to attach the eggs to the pleopods (Holdich *et. al.*, 1978). They are then carried under the abdomen of the female throughout the dormant over-wintering period until they hatch. Hatching, like moulting, appears to be temperature dependent. It was observed to occur at the end

of June and beginning of July for both years studied. In Markfield Quarry no 0+ juveniles were ever caught, but egg remains were first observed on the pleopods of the females in July, although one animal was caught which still had one egg attached. Thus it appears that hatching occurs in both populations at about the same time, but possibly slightly later at Markfield Quarry. The juveniles remain attached to the female even after the first moult. They cling to the pleopods using their chelae, but soon become free living. Up to seven moults may occur prior to the first over-wintering period, and then this number is reduced with size and age. *A. pallipes* may live for up to 10 or 11 years in the Midlands (see Tables 5.6, 5.7).

DISCUSSION

In the northern hemisphere all crayfish belong to the Astacidae, which is composed of the Astacinae and Cambarinae (Huxley, 1896; Kaestner, 1970). *Austropotamobius pallipes* belongs to the Astacinae, along with all other European crayfish, and the North American *Pacafastacus* species. The life histories of the two sub-families have been compared and reviewed by Brown (1979). Considerable variations between species of the two sub-families were reported, but within the Astacinae the life histories of different species are very similar.

A. pallipes has a typical Astacine life cycle, consisting of a summer growth and moult period after which eggs may be produced in the autumn. They are carried on the maternal pleopods over the winter, which is a dormant period during which no growth occurs. Hatching occurs early during the following summer (e.g., cf. *Astacus astacus*: Abrahamsson, 1971; Kossakowski, 1971.

Astacus leptodactylus: Kossakowski, 1971. *Pacifastacus leniusculus:* Abrahamsson, 1971; Mason, 1974). Any differences which occur between members of the Astacinae tend to be minor, and relate to details such as the timing of events due to differing environmental conditions.

Table 3.1 summarizes the timing of events in the life history of *A. pallipes* for several populations in Britain. It may be seen that the growing season starts the earliest for the two most southerly populations (R. Darent, Thomas and Ingle, 1971; R. Ouse, Pratten, 1980), and also lasts for longer. The northerly population (Northumberland aqueduct, Brown, 1979) has the shortest growing season, and it both starts later and finishes earlier than the others. Considering the two Midlands populations, the River Leen is similar to the southerly populations, whilst Markfield Quarry is intermediate. These regional population differences are explained by temperature variations which are of primary importance in regulating growth and the timing of moulting (see 5.1). The differences between the two Midlands populations lie in the fact that since Markfield Quarry is a large water body, it takes longer to increase in temperature than the River Leen. It must also be noted, however, that the method of trapping animals was biassed towards large crayfish, and towards males. This could form an alternative explanation to the observation of later moulting at this site, since the smaller animals which tend to moult the earliest also tend not to be caught. It may also explain the result that a high proportion of mature females from Markfield Quarry had moulted at the start of the growing season, which was not found to be the case in the River Leen. The moult frequencies

which relate to the different sexes and sizes of crayfish are similar to those reported for *A. pallipes* elsewhere in Britain (Brown and Bowler, 1978; Pratten, 1980). Other aspects of the environmental conditions which also affect growth and moulting, such as photoperiod and food availability, are discussed more fully in Chapter 5.

A similar relationship to that shown for moulting in the three areas of Britain exists also for the timing of egg laying and hatching. They occur the earliest in the south of England, next in the two Midlands populations with Markfield Quarry slightly later than the River Leen, and finally in Northumberland. Again this appears to be a temperature dependent difference. Photoperiod has been shown to affect the breeding cycle of *Cambarus* species (Stephens, 1955), but it is not certain whether the Astacinae are similarly affected.

Differences observed between populations for the age at sexual maturity relate to the differing growth rates, and therefore the different times required to reach the minimum size at which sexual maturity may occur. Thus, in the southerly populations where the growth rate is faster, some animals may be sexually active by the autumn of their third year (2+ age class), although the majority will not produce eggs until the fourth year. This is also the situation for the River Leen population. Those of Markfield Quarry at the Northumberland aqueduct, however, do not reach sexual maturity until their fourth year (3+ age class) since the slower growth rates for these populations (see 5.1) mean that at the end of the third year they are still too small to produce eggs. The fact that the majority of egg bearing females

were greater than 30 mm (C.L.) and therefore in an additional age class and above was also reported. The differing proportions of small animals bearing eggs observed between the three sites may be related to population density, since food availability and population density affect fecundity, and will affect the smaller size classes the most (see 3.2(ii)).

Male crayfish may become sexually active at a smaller size than the females. Thomas and Ingle (1971) report that a male of 23 mm (C.L.) was observed to pass spermatophores, and Brown (1979) reports a size of 22 mm (C.L.). Similarly, however, as is the condition observed for females, the majority of males probably do not become sexually active until they are larger. In this study 25 mm is taken as a convenient approximate minimum size at maturity for both sexes.

TABLE 3.1 A SUMMARY OF THE LIFE HISTORY OF *A. PALLIPES* IN BRITAIN, TIMING OF EVENTS

LOCATION	MOULT/GROWING SEASON		TEMP. RANGE IN GROWING SEASON	FERTIL-IZATION	EGG LAYING	TEMP. WHEN EGGS LAID	HATCHING	TEMP. AT HATCHING	AGE AT SEXUAL MATURITY	REFERENCE
	BEGINS	FINISHES								
RIVER LEEN, (MIDLANDS)	LATE MAY (1980, 1981)	LATE OCTOBER (1980, 1981)	10-20°C	OCTOBER	EARLY NOVEMBER	4.5°C	LATE JUNE TO EARLY JULY	~20°C	2+ - 3+	AUTHOR
MARKFIELD QUARRY (MIDLANDS)	MID JUNE (1981)	LATE SEPT-EMBER (1981)	11-17°C	OCTOBER	ASSUME NOVEMBER	9°C	EARLY JULY	14°C	3+	AUTHOR
RIVER DARENT (SOUTH)	MID MAY	LATE OCTOBER	11-21°C	LATE SEPT-EMBER	EARLY NOVEMBER	~5°C	MID JUNE	~ 18°C	2+ - 3+	THOMAS AND INGLE, 1971
RIVER OUSE (SOUTH)	MAY	OCTOBER	10-24°C	-	-	-	JUNE	~ 13°C	2+ - 3+	PRATTEN, 1980
NORTHUMBER-LAND AQUEDUCT (NORTH)	LATE JUNE (1974-1976)	EARLY SEPT-EMBER (1974-1975)	10-17°C	MID OCTOBER	MID NOVEMBER	-	LATE JULY AND EARLY AUGUST	-	3+	BROWN, 1979
WHITE LAKE, (IRELAND)	LATE JULY (1969)	SEPT-EMBER (1969)	15-20°C	SEPT-EMBER	NOVEMBER	<15°C	JUNE	14-21°C	2+ - 3+	MORRIARTY, 1971

TABLE 3.2 TO SHOW THE PROPORTION OF ANIMALS IN EACH MONTH OF THE GROWING PERIOD WHICH HAVE UNDERGONE AT LEAST ONE MOULT, FOR DIFFERENT SUBPOPULATIONS (±25 mm CL)

POPULATION	SUB-POPULATION	MOULT CONDITION	PROPORTION MOULTED, OR UNMOULTED, IN MONTH;					
			JUN	JUL	AUG	SEP	OCT	NOV
RIVER LEEN (1980)	TOTAL MALES	MOULTED NOT MOULTED	65.9 34.1	97.4 2.6	95.8 4.2	100.0 0.0		
	MALES ≥ 25 mm	MOULTED NOT MOULTED	38.1 61.9	94.4 5.6	92.0 8.0	100.0 0.0		
	MALES < 25 mm	MOULTED NOT MOULTED	95.0 5.0	100.0 0.0				
	TOTAL FEMALES	MOULTED NOT MOULTED	37.3 62.7	70.5 29.5	85.1 14.9	87.0 3.0	100.0 0.0	
	FEMALES ≥ 25 mm	MOULTED NOT MOULTED	7.4 92.6	48.6 51.4	65.0 35.0	94.7 5.3	100.0 0.0	
	FEMALES < 25 mm	MOULTED NOT MOULTED	76.2 23.8	100.0 0.0	100.0 0.0			
RIVER LEEN (1981)	TOTAL MALES	MOULTED NOT MOULTED	96.6 3.3	100.0 0.0				
	MALES ≥ 25 mm	MOULTED NOT MOULTED	88.2 11.8	100.0 0.0				
	MALES < 25 mm	MOULTED NOT MOULTED	100.0 0.0	100.0 0.0				

TABLE 3.2 TO SHOW THE PROPORTION OF ANIMALS IN EACH MONTH OF THE GROWING PERIOD WHICH HAVE UNDERGONE AT LEAST ONE MOULT, FOR DIFFERENT SUBPOPULATIONS (±25 mm CL) Continued

POPULATION	SUB-POPULATION	MOULT CONDITION	PROPORTION MOULTED, OR UNMOULTED, IN MONTH;					
			JUN	JUL	AUG	SEP	OCT	NOV
RIVER LEEN (1981) (continued)	TOTAL FEMALES	MOULTED NOT MOULTED	46.7 53.3	66.7 33.3	97.3 2.7	93.9 6.1	95.0 5.0	100.0 0.0
	FEMALES ≥ 25 mm	MOULTED NOT MOULTED	12.5 87.5	40.0 60.0	94.7 5.3	93.3 6.7	92.9 7.1	100.0 0.0
	FEMALES < 25 mm	MOULTED NOT MOULTED	83.3 16.7	100.0 0.0				
MARKFIELD QUARRY (1981)	TOTAL MALES	MOULTED NOT MOULTED	62.2* 37.8	72.4 27.6	100.0 0.0			
	MALES ≥ 25 mm	MOULTED NOT MOULTED	56.2* 43.8	72.4 27.6	100.0 0.0			
	MALES < 25 mm	MOULTED NOT MOULTED	100.0* 0.0	100.0 0.0				
	TOTAL FEMALES	MOULTED NOT MOULTED	75.8 24.2	29.4 70.6	77.8 22.2	92.9 7.1	100.0 0.0	
	FEMALES ≥ 25 mm	MOULTED NOT MOULTED	70.4 29.6	29.4 70.6	77.8 22.2	92.9 7.1	100.0 0.0	
	FEMALES < 25 mm	MOULTED NOT MOULTED	100.0 0.0					

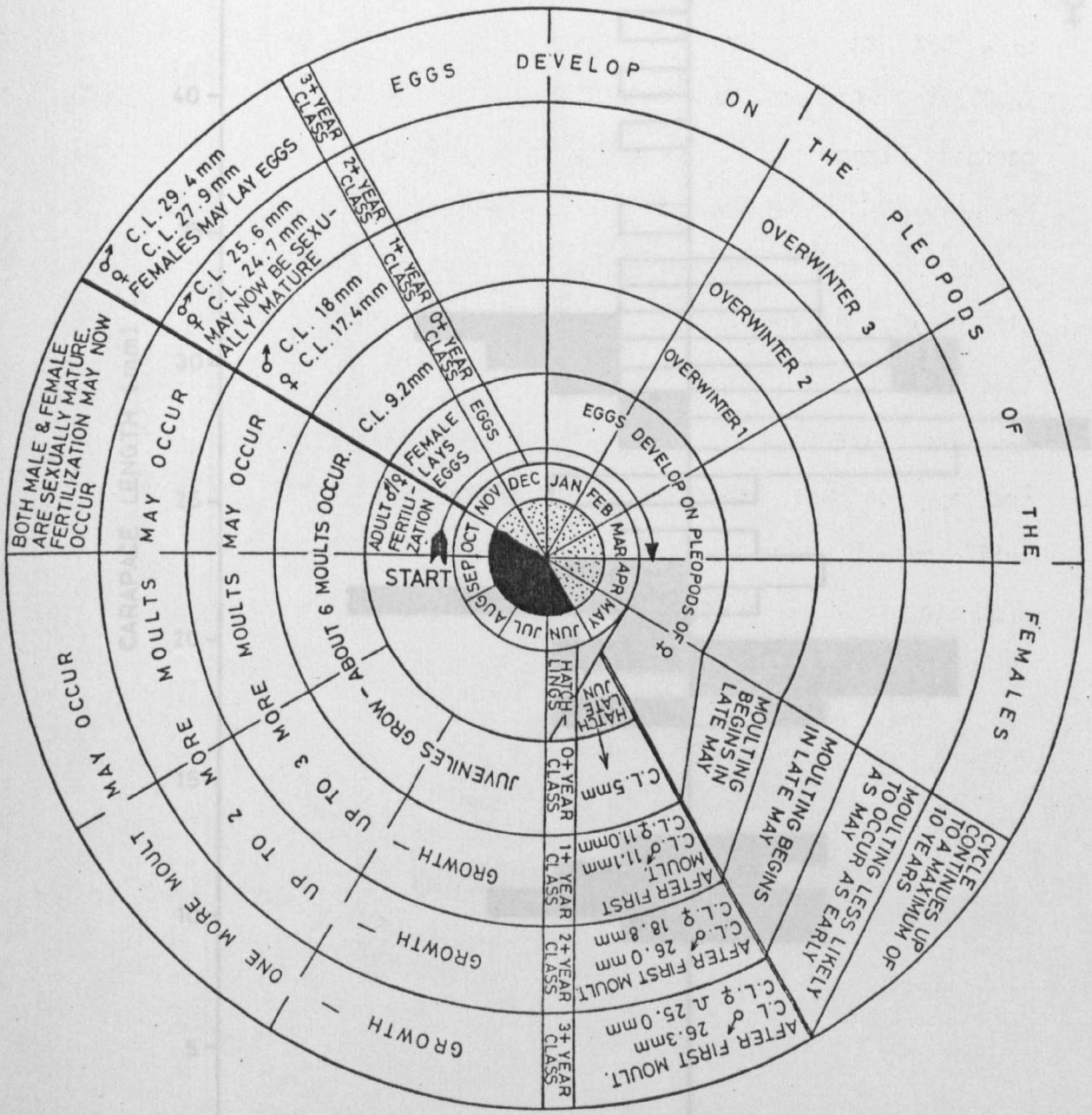
* Result based on Snorkell collection

FIG. 3.1

This figure illustrates an idealized life history for *A. pallipes*. It is constructed using data collected from the River Leen population during 1980, and so for other years and other populations the precise timing of events may differ. The size and age at sexual maturity is based on the fact that the smallest ovigerous female caught measured 23.3 mm (C.L.). The carapace lengths (C.L.) given are approximate, and are based on polymodal size frequency analysis (see 5.1). Moult frequency is calculated from analysis of frequency data in conjunction with moult increment analysis from recaptured moulted animals (see 5.1).

The life history traces one generation of crayfish from the egg on the maternal pleopods, through three year classes until sexual maturity when that generation may itself start reproducing.

Fig. 3.1



KEY:-

GROWTH / MOULTING PERIOD

DORMANT OVERWINTER PERIOD

FIG. 3.2(a) SIZE FREQUENCY HISTOGRAM TO ILLUSTRATE THE NUMBER AND SIZE OF RIVER LEEN CRAYFISH WHICH HAVE MOULTED BY JUNE 1980 THE START OF THE GROWTH PERIOD

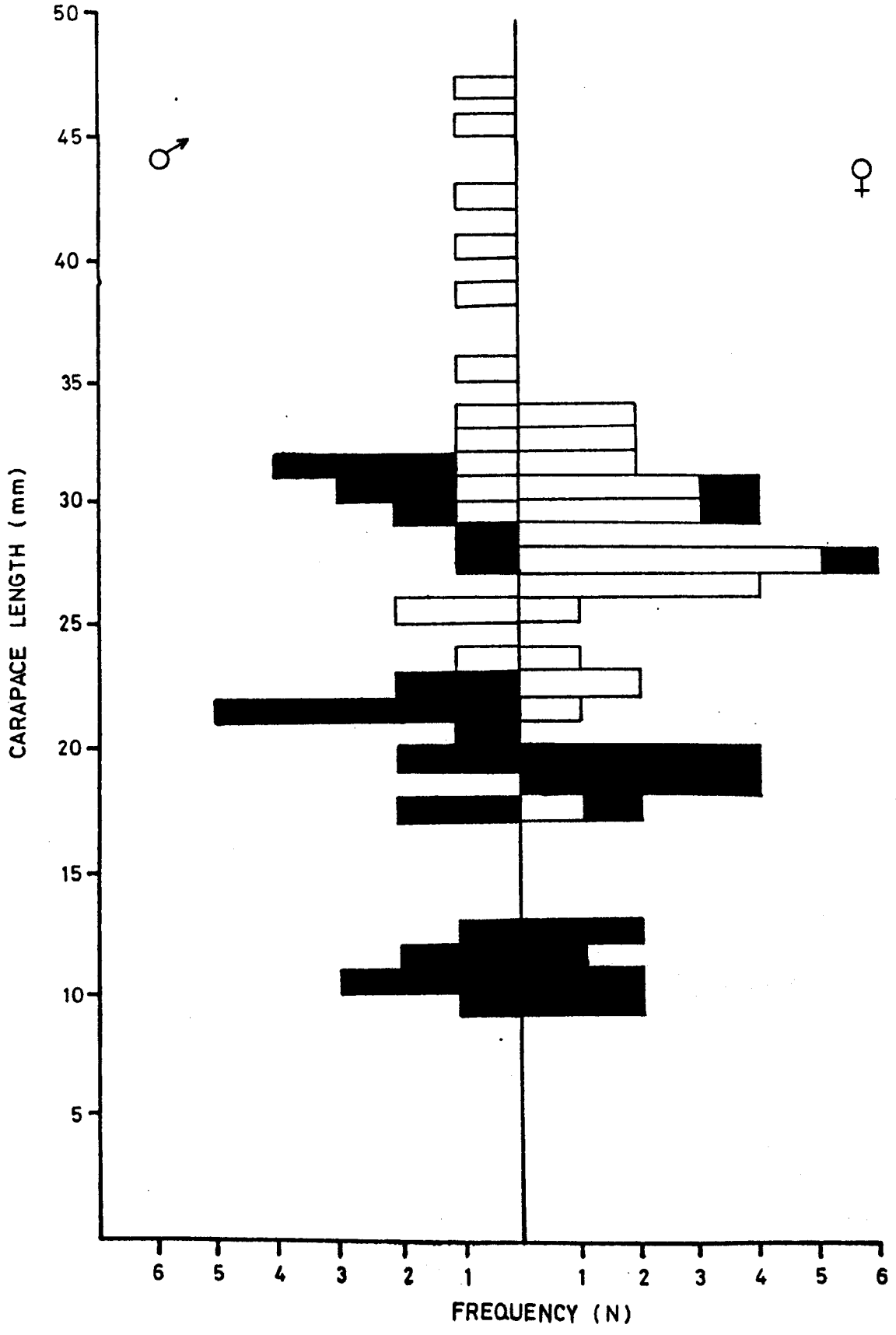
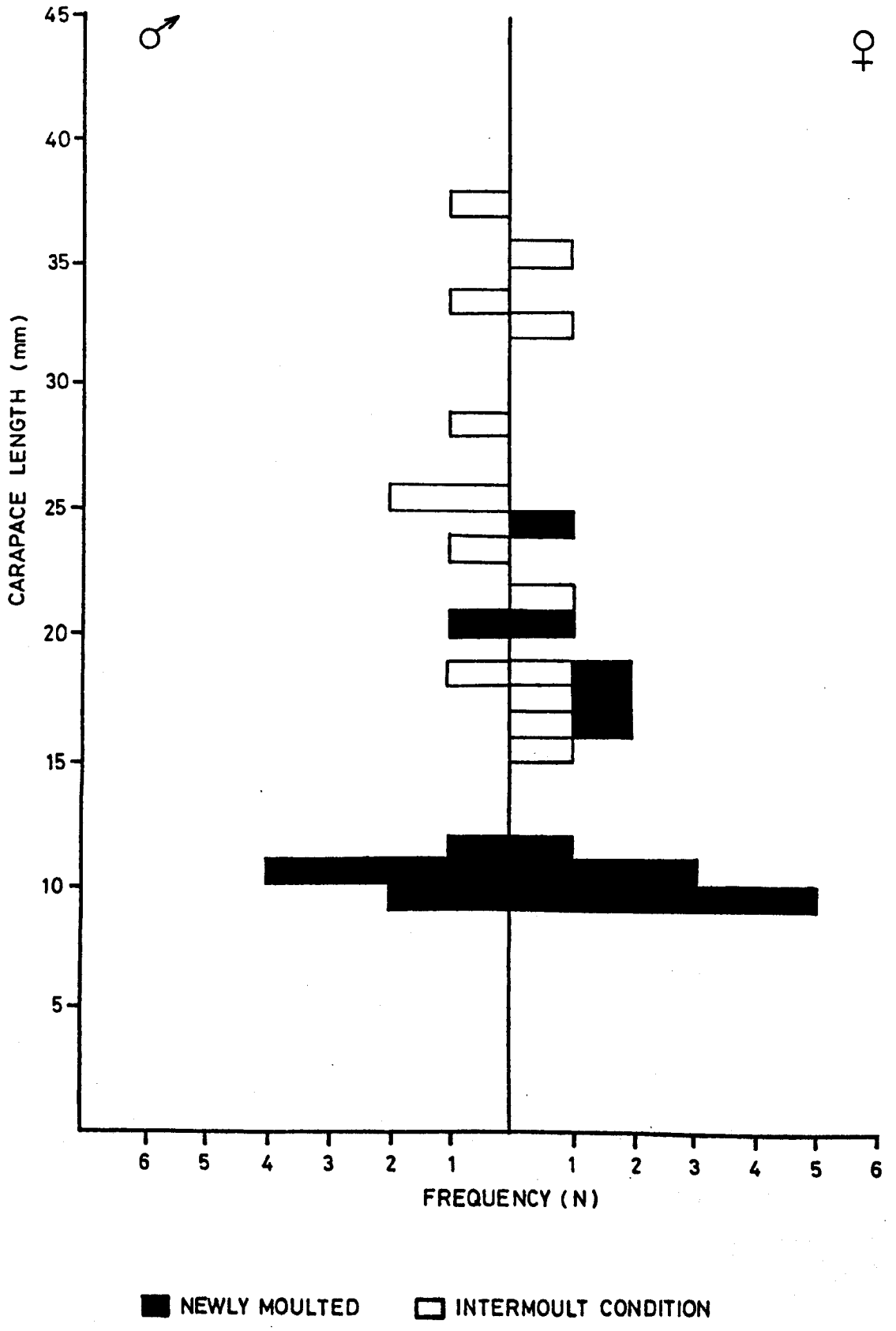


FIG. 3.2(b) SIZE FREQUENCY HISTOGRAM TO ILLUSTRATE THE NUMBER AND SIZE OF RIVER LEEN CRAYFISH WHICH WERE STILL MOULTING AT THE END OF THE 1980 MOULTING SEASON



3.2(ii) THE FECUNDITY OF MIDLANDS POPULATIONS

METHODS

All ovigerous females caught from the River Leen during the two year study period were subject to the usual measurements and marking prior to their return to the River. A count was also made of the number of eggs attached to the pleopods, which was repeated three times to ensure accuracy. In total, 28 females carrying eggs were caught between November 1979 and June 1980 (i.e. 1980 data), and 21 were caught between the same months in the 1981 period. In addition, hatching was observed to have occurred by July, when, in 1980, three females were caught with egg remains attached to their pleopods, and five with juveniles. In 1981 9 females had juveniles attached. Data were analysed both separately (for the two years), and combined.

Ovigerous females from Markfield Quarry were treated similarly. However, due to the 'trap shyness' (see 2.1) of females very few ovigerous females were caught from this site. A snorkell expedition aimed specifically at providing ovigerous females for juvenile stocking purposes was conducted in June 1981, but only one egg bearing female was caught on this occasion. In total eight ovigerous females were caught. Two of these, caught in July had only four eggs between them, so some may have hatched. Consequently these animals were not included in the analysis. Evidence of egg remains were found on the pleopods of two other females caught during July.

In addition to the collections from the two study areas, many ovigerous females were caught from Nanpantan reservoir in November 1979. Unfortunately, due to the large number of animals

to be processed, accurate egg counts were not made immediately, although the presence of eggs and a rough estimate of their numbers was recorded. A total of 180 ovigerous females were caught. In May 1980, 75 of these were transferred to individual containers (see plate 3.1), each with its own water supply and hide. This was in order to obtain juvenile stock for experimental purposes, but as a by product of doing so, it was decided to monitor egg numbers monthly. They were hence counted accurately each month until hatching, and details are presented on the fecundity of the Nanpantan stock.

RESULTS·

The minimum size at sexual maturity for female crayfish is generally gauged by observations of the smallest females bearing eggs. In the River Leen one female of only 23.3 mm (C.L.) was caught during June 1981, and it had 17 eggs. However, the majority of animals tended to be of at least 25 mm (C.L.) and above. The smallest ovigerous females from Markfield Quarry and Nanpantan Reservoir were 29.8 mm (C.L.) and 25.0 mm (C.L.) respectively. Thus it was concluded that the size limit at sexual maturity was probably not a precise figure, but would be liable to some individual and population variation. Certainly other authors have reported slight variations in this size (see Discussion), but a suitable average concensus is about 25 mm (C.L.). It is necessary to establish this size so that analysis of the population may be divided into subpopulations of mature ($\geq$ 25 mm C.L.), and immature ($<$ 25 mm C.L.) animals, hence yielding more significant results. For example, knowledge of the proportion of females bearing eggs is of far less value than of the proportion of mature,

and therefore potentially fecund females.

Table 3.3 indicates the proportion of mature ovigerous females caught at different months of the year from each site. Brown (1979) has suggested that one reason that not all mature females bear eggs, is that they have all produced eggs in Novemnber, but that over the winter some animals may lose their broods. The results expressed in Table 3.3 relate only to very few animals and so from this alone it is not possible to state decisively whether the proportion of mature ovigerous females caught at the end of the breeding season is less than that at the beginning. To compare the populations, consideration of the mean proportion of berried females throughout the year is a more meaningful result. It may be seen that a higher proportion of females bore eggs in 1981 in the River Leen than in 1980. However, they are not significantly different from each other ($t = 1.0584$, $P > 0.2$) and the combined mean proportion of ovigerous females for the two years is 64.8 ±26.8%. This is not significantly different from the proportion at Markfield Quarry which were found to be ovigerous (55.2 ± 30.7%, $t = 0.5584$, $P > 0.5$). A similar proportion of the Nanpantan stock were found to be carrying eggs (65.45%). The mean number of eggs per female were; for the River Leen, 51 ±19 (1980), and 59 ± 22 (1981), for Markfield Quarry 37 ±18 (1981), and for Nanpantan Reservoir, recorded in 1980, 47 ±17. The maximum number of eggs counted on any one individual was 95 for the River Leen population (C.L. 35.2 mm), 61 for Markfield Quarry (C.L.= 33.8 mm) and 94 for Nanpantan Reservoir (C.L. 43.3 mm).

Although consideration of the proportion of berried females was unable to provide information on egg losses with time from

egg laying, such information was achieved from two other sources. Two marked ovigerous females from the River Leen population were captured twice. Animal number 104 (C.L. 32.4 mm) was first caught in April 1980 when it had 71 eggs. On subsequent recapture in June 1980 this number was only 50. Similarly animal number 86 (C.L. 30.0 mm) showed a loss of eggs with time, from 61 in March 1980 to 56 in May 1980. Thus it appears that egg losses do occur in the wild state. Under laboratory conditions similar egg losses were observed to occur. The 75 females of the Nanpantan stock had a mean number of 47 ±17 eggs per female in March 1980. In April this had been reduced to 38 ±16, and was only 30 ±14 eggs in May. No animals, however, were observed to lose all of their brood although some with less than 10 eggs remaining did occur (5.3%).

Despite having established that egg losses apparently occur over the winter, insufficient animals were obtained on any single occasion to enable anything but the grouping of all the overwinter data for analysis. This did not apply to the Nanpantan data for which all animals were recorded at the same time of year. It was also possible to treat the two years at the River Leen site independently. Egg numbers were plotted against carapace length (Figs. 3.3 and 3.4) and regression analyses conducted (Table 3.4).

A positive correlation is found to exist between egg numbers and increasing carapace length in each case. The result however, is insignificant for the Markfield Quarry population ($P > 0.25$) and for the 1980 data from the River Leen ($P < 0.25 > 0.1$). The explanation in the case of the former site is the low number

of ovigerous females used in the analysis (6), but for the latter, the lack of significance must be explained by the high variation in egg counts observed that year. This was particularly apparent in the larger females where both low and high egg counts were observed (see Fig. 3.3). Both the analyses for the Leen 1981 data, and Leen combined data proved to be highly significant, as were those of the Nanpantan data ($P<0.01$).

Comparing the slopes of the regression analyses it is found that the Leen 1980 and 1981 data differ significantly from each other ($t = 2.3330$, $P<0.025>0.01$), but neither differs from the combined data ($P>0.1$). No difference was observed between the Leen 1980 data and either that of Nanpantan ($P>0.5$) or Markfield Quarry ($P>0.5$). The 1981 data, however, showed significantly greater egg counts with increasing carapace length when compared with Nanpantan ($t = 4.5808$, $P<0.001$), though not with Markfield Quarry ($P>0.5$). The situation when comparing the combined Leen data was similar, showing significant differences between the River Leen and Nanpantan females ($P<0.025>0.01$), but none with Markfield Quarry ($P>0.5$). Comparing Nanpantan with Markfield Quarry no significant differences were observed ($P>0.1$).

Thus it will be seen that more eggs are produced by females of corresponding sizes from the River Leen than Nanpantan Reservoir. That no differences were observed between either population and Markfield Quarry is a reflection of the small number of animals caught at that site resulting in an insignificant regression analysis.

Having examined the fecundity of the population, it is possible to estimate the potential recruitment. It is necessary to know

the population size, and the proportion of mature, berried females. These details are provided in 4.1 and 4.2, and combined with knowledge of the mean egg count per female, recruitment may be estimated.

For the River Leen in 1980, the Jolly (1965) model estimated a total population of 1,071 (4.1), in the study area. The ratio of immature:mature animals was found to be 0.53:0.47, and the sex ratio of mature animals was 0.89 (4.2). Hence there were 503 mature animals of which 266 were female. The mean proportion of mature females which were berried was 56.6%, and the mean number of eggs per female was 51. There were therefore 151 berried females in the population study area in 1980, and the potential recruitment was 7,071. The same method is applied to the following estimates of recruitment.

YEAR	STUDY SITE	POPULATION	$\hat{N}$	% MATURE ANIMALS	SEX RATIO MATURE ANIMALS	% BERRIED FEMALES	MEAN EGG COUNT	MEAN REC RUIT-MENT
1980	LEEN	STUDY AREA	1071	47	0.89	56.6	51	7071
1980	LEEN	EAST BRANCH	3176	47	0.89	56.6	51	22798
1981	LEEN	STUDY AREA	646	46	0.98	72.9	59	6455
1981	LEEN	EAST BRANCH	1668	46	0.98	72.9	59	17461
1981	MARKFIELD	STUDY AREA	837	46	1.02	55.2	37	3893
1981	MARKFIELD	WHOLE QUARRY	15265	46	1.02	55.2	37	70998

The proportion of the Markfield population represented by mature animals was not known due to the bias of trapping towards large animals (2.1(iii)). It was therefore assumed that this value would be similar to that found in the Leen. All the estimates of recruitment are mean values, and do not take into account

any variation which occurs within each of the variables. The values for the Leen study area are thought to be reasonably accurate however, although the values for the whole river are probably gross underestimates due to the underestimation of population size (see 3.3). The estimates of recruitment at Markfield Quarry may tend to be too low also, since a surprisingly low egg count was achieved. This was based on only six animals, and so may not be representative of the whole population. However, observations of Markfield females which became berried in captivity, shortly after their capture, also revealed low egg counts, and so this may indeed be the true situation. Survival of the new recruitment is discussed in section 4.2.

DISCUSSION

Aspects of the fecundity of *A. pallipes* in Britain have previously been examined and reviewed by several authors (Thomas and Ingle, 1971; Brown, 1979; Rhodes, 1980; Rhodes and Holdich, 1982). Rhodes and Holdich (1982) make the point that, "possible effects of density dependent influences on the reproduction and fecundity of *A. pallipes* have yet to be investigated". They also examine two of the Midlands populations studied by this author, the River Leen, and Nanpantan Reservoir. However, data is pooled for their analysis over two years, 1977/78 and 1978/79, and so annual variations are not apparent. In the light of this, and other previous work on the fecundity of *A. pallipes* therefore, it is the intention of this author simply to briefly discuss this area of study, and highlight the factors such as the effects of population density which are still lacking.

The minimum sizes (as carapace length) observed for ovigerous females have been 25 mm in Northumberland (Brown, 1979), 28 mm in Ireland (Morriarty, 1972), 26 mm in the River Ouse (Pratten, in Brown, 1979), 27 mm in the River Darent (Thomas and Ingle, 1971), 23.1 mm (Rhodes, 1980; Rhodes and Holdich, 1982) and 23.3 mm (Author) in the River Leen, 28.2 mm (Rhodes, 1980; Rhodes and Holdich, 1982) and 25 mm (Author) in Nanpantan Reservoir, and 29.8 mm at Markfield Quarry (Author). Some population variation therefore seems to exist, and this may be related to differences in the growth rate. If sexual maturity is related to size rather than age, which it appears to be from a comparison of both age and size at maturity (see 5.1), then in slow growing populations such as that in Northumberland (Brown, 1979) the minimum size at which eggs have been reported (23.1 mm) will not have been reached by the breeding season in one year (the second in Northumberland, Brown, 1979), but will have been exceeded by the following year, hence a larger size is reported for sexual maturity. Similarly in populations such as that in the River Darent, the minimum size will have been exceeded by the breeding season, again indicating a larger minimum size at sexual maturity than was observed in the Leen. Alternatively, genetic differences between geographically isolated populations may be the explanation. The reason for the large size reported for the Markfield Quarry population is due to the small number of ovigerous females caught and probably smaller animals are capable of bearing eggs.

Not all animals of the minimum size and above will be ovigerous and the larger the carapace length, the greater the tendency to produce eggs (Brown, 1979). The frequency of spawners does not usually reach 100% until one or two years after the minimum size at

sexual maturity in Marron *Cherax* (Morrissy, 1970), and a similar situation may exist for *A. pallipes*. In addition, a positive correlation exists between carapace length and egg numbers. This was found to be the case in this study, and also in those reported above. It is also the case for other crayfish species (see Brown, 1979, for review) and Rhodes and Holdich (1982) argue further, that the correlation is between the body volume of the female, and the number of eggs. The easiest way to appreciate the correlations found for each population discussed, is to consider the predicted pleopod egg counts for particular sizes (C.L.);

SITE	TIME	POP-ULATION DENSITY	PREDICTED EGG NO'S AT:		REFERENCE
			27mm(CL)	42mm(CL)	
RIVER DARENT (SOUTH)	1963-1964	-	70	130	THOMAS AND INGLE, 1971
NORTHUMBERLAND AQUEDUCT (NORTH)	1975-1978	4-10m^{-2}	5	124	BROWN, 1979
RIVER LEEN (MIDLANDS)	1977-1979	-	41	108	RHODES (1980); RHODES AND HOLDICH, (1982)
RIVER LEEN	1979-1980	3.6m^{-2}	43	73	AUTHOR
RIVER LEEN	1980-1981	2.2m^{-2}	43	109	AUTHOR
NANPANTAN RESERVOIR (MIDLANDS)	1977-1979	-	16	84	RHODES, (1980); RHODES AND HOLDICH, (1982)
NANPANTAN RESERVOIR	1979 (NOV)	-	31	58	AUTHOR
MARKFIELD QUARRY (MIDLANDS)	1980-1981	6-8m^{-2}	26	77	AUTHOR

The sizes 27 mm and 42 mm were chosen because Thomas and Ingle (1971) did not correlate egg number to size thus producing a predictive model, as was done for all other populations cited. Instead they gave the egg counts observed for animals of those sizes, which are reported above for comparison. It may be seen that females of the Darent population produce the greatest number of eggs at both sizes. The Northumberland animals of small carapace length produce the least number of eggs whilst larger animals are comparable to the Darent population. None of the Midlands populations are so extreme, and produce an intermediate number of eggs at the smaller size, and fewer at the larger size, when compared with the northerly and southerly populations. In this study a significant difference was also observed between the Leen and Nanpantan crayfish, the former tending to produce more eggs for any given size. The Markfield population was intermediate, but the correlation was not significant.

Comparing the results of Rhodes (1980), and Rhodes and Holdich (1982) with those from this study it is found that for the River Leen the pooled values from 1977-1979 were almost exactly the same as the 1980/81 data, and both were different from the 1979/80 data. The Nanpantan results also differed from each other. This may perhaps be explained by the fact that those relating to this study are based upon laboratory held animals. Thus the situation is artificial in that excessive crowding of the population, resulting in some egg losess, may have occurred prior to separating the ovigerous females into individual containers.

This study concluded that egg losses occur with time from egg laying. Rhodes and Holdich (1982) noted that egg losses occur

under laboratory conditions which they attributed to stress. However, they concluded that no losses occurred in the wild with increasing time. In this study, although only two marked ovigerous females were recaptured, both showed a reduction in egg numbers. It was also the conclusion of Brown (1979) that egg losses occurred over the winter, and Payne (1978) suggests that a significant reduction in egg numbers may occur due to their removal by knocking against obstacles. The lower pleopodal egg counts which are seen to occur when compared with ovarian egg counts (Momot, 1967; Rhodes, 1980; Rhodes and Holdich, 1982) are explained by such losses, and also due to failure of eggs to extrude or attach themselves (Payne, 1978). For *P. leniusculus* it is reported that a 40-50% egg loss prior to hatching is not unusual (Mason, 1974), and Kossakowski (1971) reported that overwintering egg losses amongst the Astacinae are common.

The loss of eggs over the winter has two important consequences. The first is that the predictive models for the Midlands populations are based on data collected throughout the whole breeding season. Since egg losses occur with time, the model will tend to underestimate the number of eggs at any given size at the time of egg laying, but will over estimate the number at hatching. This may explain some of the differences reported above. The results of Thomas and Ingle (1971) were obtained in November, directly after egg laying, when the count would be at its maximum. Similarly, Brown (1979) was able to catch 59 ovigerous animals all in the same month, two months after egg laying, and so egg losses would have been minimal at this time also. The second consequence is that egg losses may explain any observations below 100% for the

proportion of mature berried females. In this study it was not possible to see if the proportion of mature females bearing eggs decreased over the winter, and so only the mean proportion of mature berried females was given. These were 65.45% for the Nanpantan females during November 1979, 55.2 ±30.7% for Markfield Quarry (1980/81), and 56.6 ±27.8% and 72.9 ±25.5% for the Leen (1979/80 and 1980/81 respectively). These values compare with 96% during November 1964 in the River Darent (Thomas and Ingle, 1971), 41.6 ±4.2% in White Lake, Ireland (Morriarty, 1972), and 32-49% over the season in Northumberland (Brown, 1979). It may be seen that in each case the proportion of berried females was less than 100%. Kossakowski (1971) suggests that females may produce eggs only on alternate years. Brown (1979) showed that this may be the case for some individuals of *A. pallipes* in the Northumberland population, but that egg losses, and the fact that some individuals either may not have been fertilized, or were not capable of producing viable ova, explained the remaining differences from 100%.

The total population fecundity may also be examined in terms of the environmental influences upon that population. Abrahamsson (1972a) concluded that water temperature and food supply affect reproductive success. Population density is also of importance (Abrahamsson, 1966; Abrahamsson and Goldman, 1970; Morrissy, 1970). For *O. virilis* in Michigan it has been clearly demonstrated that fecundity is density dependent (Momot and Gowing, 1977 a,b,c; Momot *et. al.*, 1978), and that food availability is an important factor in egg production (Momot and Gowing, 1977 a). Individual food availability within any one population will be density dependent, and this may explain why low egg counts occurred on

small animals in Northumberland whilst the larger animals had an egg count which was comparable with the southerly River Darent. The former population has a high density (Brown, 1979), and so competition for food might be expected to exclude the smaller less dominant females.

Markfield Quarry and the Northumberland aqueduct have the greatest population densities. Both of these populations also had the lowest proportion of berried females (except for White Lake, the population size of which is thought to be an underestimate, see 4.1). The population density for Nanpantan Reservoir is not known, but it was certainly high, and the proportion of berried females from this site is intermediate between the Leen and Markfield Quarry. In fact the differences observed are not statistically significant, but in real terms the values tend to suggest a trend where density is affecting fecundity.

The population density in the River Leen study area was calculated for both years of the study period, and was found to be greater in 1980 than 1981 (see 4.1). It was also found that in 1980 a smaller number of eggs were produced for any given carapace length (see Fig. 3.3), a smaller proportion of the mature females were berried, and a lower mean egg count was observed. As a consequence, the potential recruitment in 1980 was not much greater than in 1981 when a smaller population size existed. Thus it appears that a correlation exists between population density and fecundity in the Leen, and that stabilization of the population size may occur due to a density dependent feedback mechanism, which operates on the population fecundity. Comparing these results with those of Rhodes (1980), and Rhodes and Holdich

(1982) it would then suggest that the population density had increased from the 1977-79 period to 1979/80, and then decreased again in 1980/81.

The relatively low recruitment compared to population size observed in Markfield Quarry may also be a result of the high population density at this site. Such density dependent variations in fecundity are very important since they show how a population might react to fishing mortalities should it be exploited as a food resource. Momot and Gowing (1977a) showed that the population of *O. virilis* in a Michigan Lake was in fact well able to cope with the stress imposed by fishing mortalities, and it appears that this may also be the case with *A. pallipes*. Compensation was made in the form of increased juvenile survivorship, and increased egg production, providing that overfishing did not occur (Momot and Gowing, 1977c).

TABLE 3.3 TO SHOW THE PROPORTION OF BERRIED ADULT (≥25 mm C.L.) FEMALES CAUGHT ON ANY PARTICULAR MONTH FOR EACH POPULATION

LOCATION	TIME	NO. BERRIED	TOTAL ADULT ♀	% ADULT ♀ BERRIED
NANPANTAN	NOV. 1979	180	271	65.45%
MARKFIELD QUARRY	FEB. 1981	1	1	100.0%
	APRIL 1981	1	3	33.3%
	MAY 1981	3	8	37.5%
	JUNE 1981	2	4	50.0%
	JULY 1981	1 (2 E.R.)	17	5.8% (11.8%)
				MEAN = 55.2% ±30.7
RIVER LEEN	JAN. 1980	2	2	100.0%
	FEB. 1980	2	3	66.7%
	MAR. 1980	3	6	50.0%
	APR. 1980	2	4	50.0%
	MAY 1980	1	7	14.3%
	JUNE 1980	17	29	58.6%
	JULY 1980	8 (E.R.)	35	(22.9%)
				MEAN = 56.6% ±27.8
RIVER LEEN	NOV. 1980	5	10	50.0%
	JAN. 1981	1	1	100.0%
	MAR. 1981	1	2	50.0%
	APR. 1981	2	4	50.0%
	MAY 1981	1	1	100.0%
	JUNE 1981	7	8	87.5%
	JULY 1981	9 (JUVS)	21	(42.9%)
				MEAN = 72.9% ±25.5%

E.R. = Egg remains present.

TABLE 3.4 THE RESULTS OF THE REGRESSION ANALYSES OF PLEOPODAL EGG COUNTS AGAINST CARAPACE LENGTH

(EGGS = No. of eggs, C.L. = carapace length)

POPULATION	EQUATION	S.E.b.	r	F	df	P	N
LEEN, 1980	EGGS = 1.9884 C.L. - 10.9784	1.2363	0.3008	2.5868	26	<0.25	28
LEEN, 1981	EGGS = 4.4140 C.L. - 76.6284	0.6846	0.8284	41.5708	19	<0.01	21
LEEN, 1980 + 1981	EGGS = 3.2897 C.L. - 47.6518	0.7230	0.5530	20.7059	47	<0.01	49
MARKFIELD 1981	EGGS = 3.3666 C.L. - 64.6116	3.9070	0.3957	0.7425	4	>0.25	6
NANPANTAN	EGGS = 1.8400 C.L. - 18.8261	0.5253	0.3793	12.27	73	<0.01	75

FIGS 3.3 AND 3.4

Figs. 3.3 and 3.4 show the regression of pleopodal egg counts against carapace length. The equations for these regressions occur in Table 3.4.

Fig. 3.3 relates to the Leen population and the scatter of points is illustrated. The data has also been combined to produce the average result of the 1980 and 1981 data. This line is also illustrated in Fig. 3.4 (broken line) by means of comparison with the Markfield and Nanpantan data. The Markfield data was comprised of only six animals as indicated in the figure. For the Nanpantan data note particularly the great scatter of egg counts observed over the range of different carapace lengths.

FIG. 3.3 TO SHOW THE RELATIONSHIP BETWEEN PLEOPODAL EGG COUNT AND CARAPACE LENGTH FOR THE LEEN POPULATION DURING 1980 (●) AND 1981 (▲)

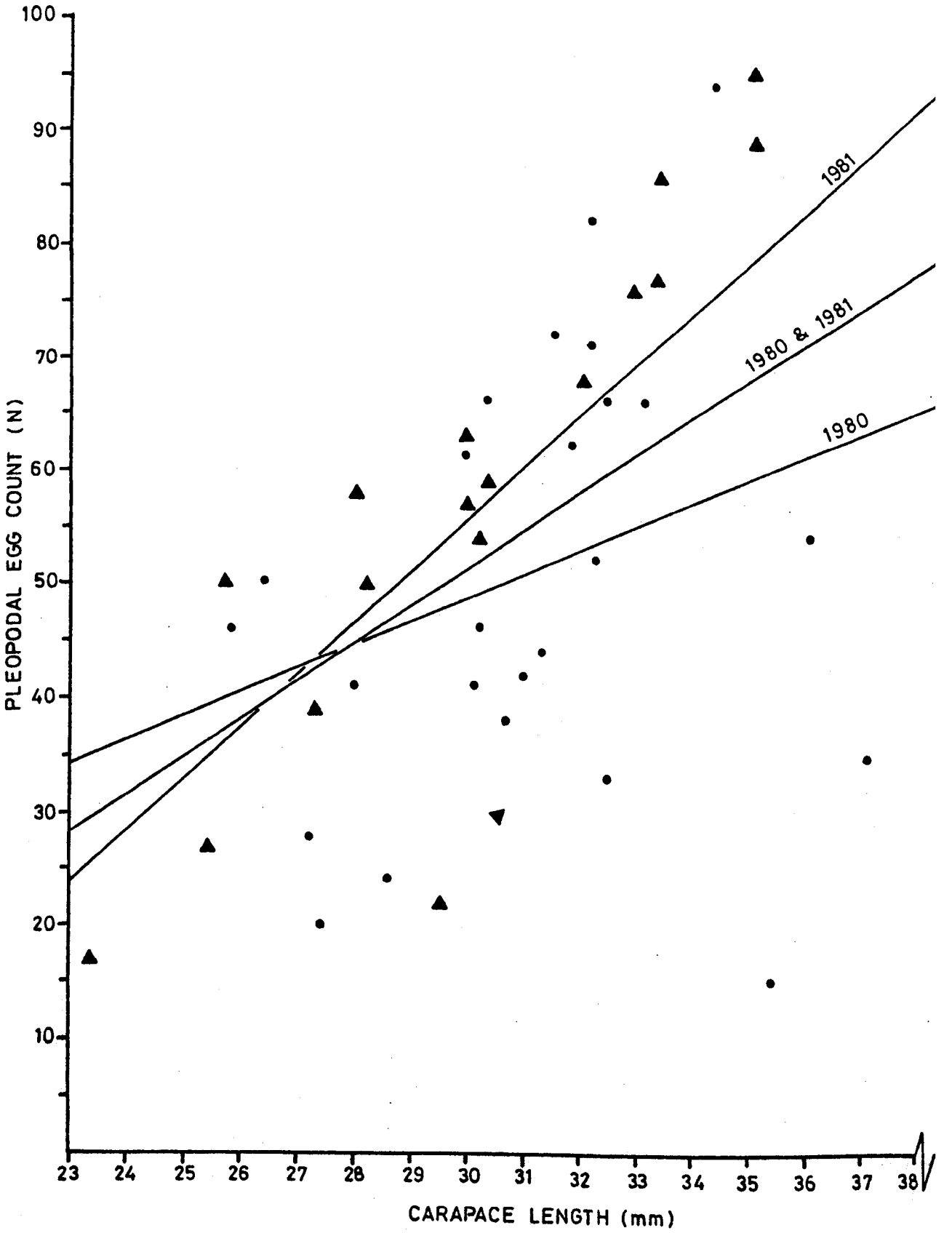
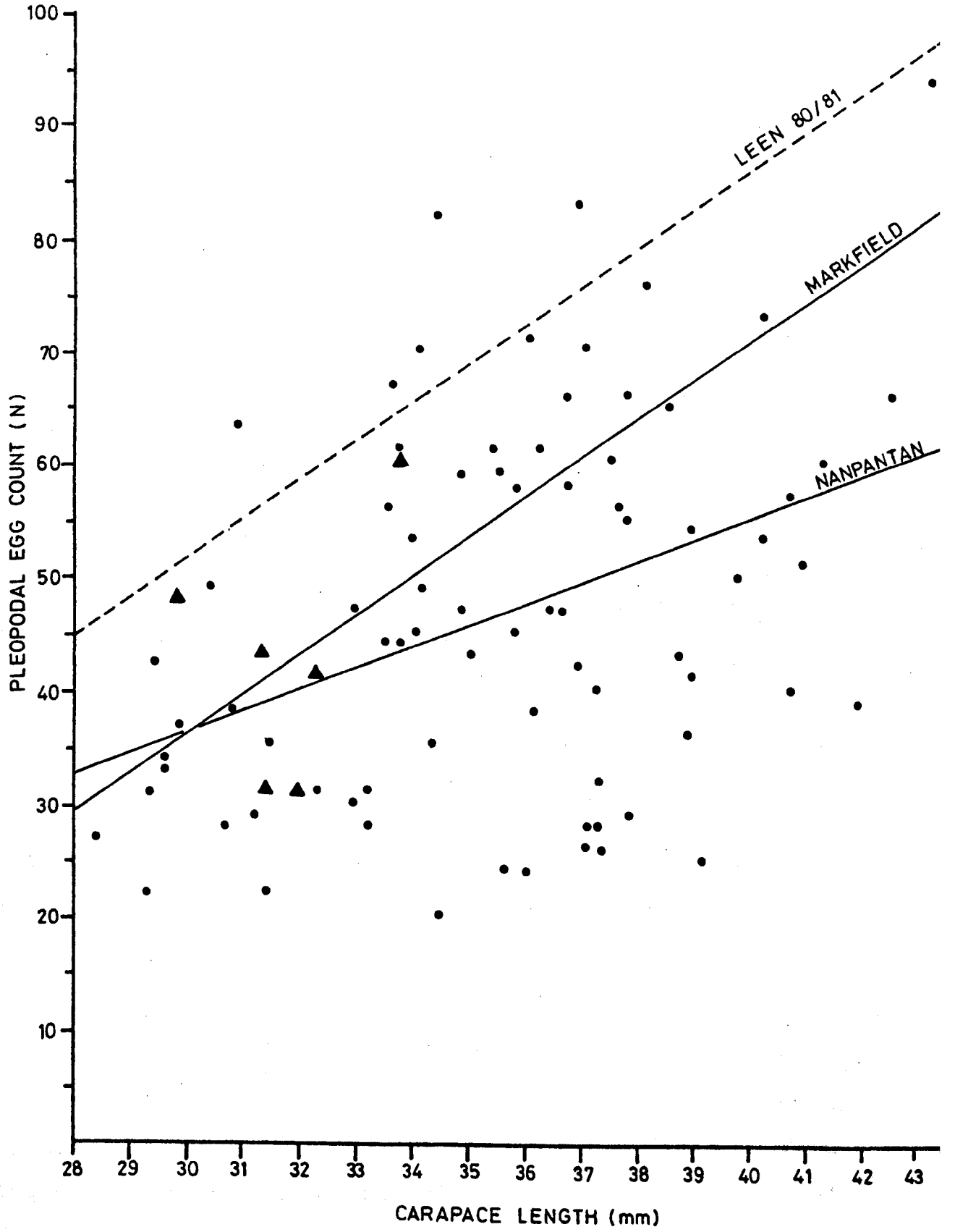


FIG. 3.4 TO SHOW THE RELATIONSHIP BETWEEN PLEOPODAL EGG COUNT AND CARAPACE LENGTH FOR THE NANPANTAN (•) AND MARKFIELD (▲) POPULATIONS



3.3 THE LOCAL DISTRIBUTION AND MOVEMENT OF CRAYFISH

3.3(i) THE RIVER LEEN

During the period 18-22 August 1980 the author surveyed the full length of the River Leen to record any local distribution differences of crayfish along its length. Figure 3.5 shows the upper reaches of the river, and both east and west branches. The numbers to the right of the river indicate the relative abundance of crayfish on a scale of 0-4. '0' indicates that they are absent, '1' that they occur, and then as the scale increases to 4 it indicates that crayfish are becoming more abundant. This was based on subjective observations relating to the numbers of crayfish found within approximately equal searching times. To the left of the river on the map (Fig. 3.5) are the letters A-F. These refer to the major collecting areas discussed below. Further collections were made below the confluence of the two branches, and these relate to the following; from the confluence to the B6001, from the B6001 to Papplewick Lido, then subsequent areas approximately equivalent to the S.T.W.A. sampling sites numbers 3, 4 and 5 (see Fig. 1.1).

Area A on Fig. 3.5 represents the beginning of the river. At this point its width varies between one and two metres and the substrate consists of fine mud and sand with a few rocks and stones. No crayfish were observed. The ponds illustrated are very silt laden, and the uppermost had been drained at the time it was visited in 1980. No dead crayfish were seen on the sides of the pond. All the three lakes in area B were very silt laden. The upper two occur in the grounds of Newstead Abbey, and the interconnecting part of the stream forms part of an

ornamental garden. It has a firm substrate at this point with small stepping stones and man-made weirs being amongst its features. However, no crayfish were observed either here, or in the two lakes. Indeed, every two or three years the Garden Lake is dredged due to the build up of decaying organic matter after the summer's growth of 'blanket weed' (*Cladophora*) falls to the bottom. Mr. Lithland (pers. comm.), who has been the head gardener for over 17 years, reports that on these occasions he has never seen any crayfish and neither has he heard any reports from fishermen of crayfish in the lake.

From the Garden Lake outside the Newstead Abbey grounds to the Lower Lake the river is again very silt laden and has few rocks and stones in it. No crayfish were observed here, nor were they seen when using SCUBA in the Lower lake during a previous visit in June 1980. At the visit made in August it was found that the lake had been completely drained and the river was rerouted along its original course, shown by the broken line. No crayfish remains were to be found, and the deep mud substrate had no rocks suitable for hides.

Beneath the Lower Lake the substrate of the Leen changes and becomes more firm, consisting of sand and gravel with many rocks present. This is area 'C'. The river continues to broaden out from about two metres to five metres wide at the population study site, the weirs of which are represented by the closed circles. All along this length of the river the substrate, although 'patchy', remains sand and gravel based. The number of rocks and large stones present increases from Lower Lake to the study area, and it is this area which is observed to have the most

crayfish. Directly beneath the lake some juveniles were caught and so a weir was constructed in the hope of increasing the catch from this area. Towards the ford crayfish became more abundant, and below it this trend continued, with the greatest number of crayfish being observed in the population study area. The substrate in this area has been described previously (see 1.1) and illustrated in Fig. 1.2. It is interesting to note that the only crayfish found in the sandy area prior to the weirs were all beneath a few large rocks in a localized area near the banks of the river.

Below the study area and footbridge, the substrate becomes increasingly muddy and silt laden once again (Area D). This is probably due to the construction of permanent weirs both prior to and after the confluence with the west branch. In this area the author had constructed a fifth weir and it proved to be the only part of area D where crayfish were caught.

Area E, below the permanent weirs, again has a firm substrate, but there are fewer rocks present. This area is now below the confluence of the two branches of the Leen. A quick survey of the west branch revealed no crayfish although they have been reported there (Saleem, 1980), and in Area E only one crayfish was found after considerable searching. This animal was in fact out of the water and making considerable progress upstream along tree roots!

The small pond shown on the map at the start of Area F is now empty, and then below this point the Leen becomes increasingly deeper (about 1 m) and silt laden. Flowering aquatic plants and reeds also grow at the sides of the river. No crayfish were caught here, nor at any of the other points downstream towards

the Trent, although crayfish have been reported at Papplewick Lido, just below the B6001 (Jones, pers. comm.).

Thus it may be seen that the population of crayfish in the Leen is confined chiefly to the East branch of the river between a point just below what was originally Lower Lake, and the confluence with the West branch. This stretch of river covers approximately two miles and was surveyed more thoroughly by the author and his colleagues in both September 1980, and August 1981. The area was divided into 8 sections, described in Table 3.5 (see also Fig. 3.5), and then a collection was made of all the crayfish encountered within each section. They were individually marked and returned to the appropriate section of the river. Two days later a second catch was made and the numbers of marked animals were recorded. This enabled the calculation of a Petersen/Lincoln index population estimate (see 4.1), the results of which appear in Table 3.5. They confirm the more subjective observations of abundance, reported above, and show that the population density increases towards the population study area. The highest population recorded in each of the years studied was between the ford, and the island downstream from it. However, due to the greater length of this section compared with that of the population study area, the latter is seen to have the greatest population density. All of these estimates, however, are considered to be underestimates. This point is dealt with more fully in 4.1, but briefly it was concluded that two days may not give sufficient time for total mixing of the population, which is one of the criteria of the model. Consequently an underestimate of the population size results.

In addition to enabling such an approximate, but quantitative

rather than subjective, idea of the population size in the east branch of the Leen, these two capture programmes also enabled the assessment of any movement out of the population study area. By September of 1980 500 animals from the study area had been marked. In August 1981 this number had reached 1,000. The estimates of the size of the population within the study area were also of this order (see 4.1). Since the majority of animals caught each month were unmarked, it follows that perhaps the marked individuals may have migrated out of the study area. Very few animals bearing marks, however, were found outside the study area, and those found were within 20 metres either upstream or downstream of this site. All of those recaptured were as follows:

NO.	FIRST CAUGHT	WHERE CAUGHT	RE-CAPTURED	WHERE RECAPTURED	MOVEMENT
247	JULY 1980	WEIR (1)	SEPT.1980	WEIR (5)	DOWNSTREAM 80m
255	JULY 1980	WEIR (1)	SEPT.1980	10m UP FROM WEIR (1)	UPSTREAM 10m
331	AUG. 1980	WEIR (2)	SEPT.1980	BELOW FOOTBRIDGE	DOWNSTREAM 40m
432	SEPT.1980	WEIR (1)	SEPT.1980	10m UP FROM WEIR (1)	UPSTREAM 10m
489	SEPT.1980	WEIR (5)	AUG. 1981	WEIR (5)	NONE
668	OCT. 1980	WEIR (2)	AUG. 1981	BELOW FOOTBRIDGE	DOWNSTREAM 40m
737	NOV. 1980	WEIR (2)	AUG. 1981	WEIR (5)	DOWNSTREAM 60m

Animals recaptured after nine months have moved no further than those recaptured after one month. Indeed, animal 489 was first caught outside the population study area in September 1980, when it was marked. In August 1981 it was recaptured at the same site. No animals from outside the study area which had been marked on these two occasions were ever found in the area.

These results imply that little movement of crayfish occurs. During the study a total of 215 marked animals were recaptured. 66.05% (142) of these were recaptured at the same weir as originally, 20.47% (44) had moved downstream, and 13.48% (29) had moved upstream. Of the proportion moving downstream, 63.64% (28) had only moved 20 m (i.e. one weir), 25% (11) 40 m, 6.82% (3) 60 m, and 4.55% (2) 80 m. Similarly, the majority of animals moving upstream were observed to be only 20 m from their original site of capture (79.31% ≡ 23 crayfish), whilst 13.79% (4) had moved 40 m, and 3.45% (1) had moved 60 m, and 80 m. The average distance moved per crayfish was 28.77 m (calculated only on those that had in fact moved, and not including the 142 which had not).

These observations apply to time intervals between recaptures of as little as two days, and as much as 16 months. They support the view expressed above that very little movement of crayfish occurs. Observation of individual cases, however, reveals that considerable distances may be moved within a very short space of time. For example, animals 775, 801 and 1219 had all moved downstream 20 m in the space of two days, whilst numbers 777, 816, 864 and 909 had all moved downstream 40 m in the same space of time. Only one animal was observed to move upstream over a two day period, this being number 1238 which moved 20 m. By contrast, after long periods of time animals may still be observed at the same weirs, for example, 86 (14 months), 204 (12 months), 214 (16 months), and so on. However, this does not necessarily mean that no movement has occurred, a point that is illustrated by certain recaptures. Animal 765 was originally captured at weir (4), it then moved upstream over 5 months to weir 3, two months

later it moved upsteream again to weir (2) and then the following month returned to weir (3). No. 165 moved greater distances. It was first caught in June 1980 at weir (1), one month later it had moved 60 m downstream to weir (4), and was then recaptured two days later, again at weir (4); four months later in November 1980 it had moved upstream 60 m to weir (1), its original site of capture, and five months later it was again observed at weir (4).

Thus it appears that the observed and recorded movements of crayfish are to some extent a little conflicting. In general, however, it may be deduced that the crayfish have a home range of within 80 m, movement over a greater distance than this rarely being observed. The results imply further, that since so many animals are always found at the same site, some territoriality may exist. Examination of the proportions of males and females which have moved, however, reveals that similar numbers of each sex are displaced. The proportions were: 51% of animals remaining at the same site were males (49% females); 52.3% of those moving downstream were males (47.7% females); and 44.8% of animals moving upstream were males (55.2% females). Should territoriality exist, one would have expected that a higher proportion of those animals not moving should have been made up of males than was the case, since it is the males which tend to be dominant. This was not observed.

It has been established therefore, that the crayfish population in the River Leen is confined to the East branch, and that within this area overlapping populations have a home range of about 80 m. However, it may be asked, "what limits the crayfish to

this part of the river, given that it has been observed that they are capable of moving considerable distances in a short period of time, and could therefore potentially colonize other stretches of the river?". To attempt to answer this question, two aspects were considered. One was the substrate which, from the survey described above, appeared as though it may be a limiting factor. The second was water quality.

In order to determine the effects of substrate on distribution an artificial river was constructed at Nottingham University (see Plate 3.2). It was 0.5 m wide and 7 m long and filled to a depth of about 15 cm above the substrate, which consisted of coarse gravel along half of its length, and mud collected from the East branch of the Leen along the other half. Hides consisted of broken plant-pots and drain piping. Initially no hides were provided, and subsequently a variety of permutations were attempted with hides on mud, gravel, or both, and in greater or lesser abundance than the numbers of crayfish stocked in the river. The crayfish were added to the river at the divide between mud and gravel, and their presence was scored every two days. Table 3.6 summarizes the results of this series of experiments, and details the different hide-substrate regimes employed, whilst Table 3.7 shows analysis of these results after comparison using the t-test. Observations were generally made over a period of two weeks prior to changing the regime.

In the absence of any hides it was found that significantly more animals occurred on the gravel than on the mud ($P<0.05>0.025$). These would generally be in the corners and against the sides of the river, or else would tend to roam around. Animals in the

mud half of the river were observed to bury themselves completely. This occurred on several occasions. When hides were added, (initially in equal numbers to the number of crayfish and half on each of the mud and gravel) it was found that a highly significant number of animals selected hides in preference to remaining unhidden ($P<0.001$). Furthermore, it was found in this case, that significantly more animals chose hides on the mud ($P<0.025>0.01$) than the gravel, and similarly mud was preferred by those animals not selecting hides ($P<0.05>0.025$). Next the number of available hides was doubled, and also six more crayfish were added. Hence an excess of hides was available, but still evenly distributed about the mud and gravel. In this case all the animals were observed to select hides on every occasion, and no significant difference was observed between the choice of hides on either substrate ($P>0.2$).

Next, equal numbers of hides and crayfish were used, but all the hides were placed on the mud. It was again found that a highly significant preference occurred towards the occupation of hides ($P<0.001$), and that of those animals not selecting hides, all were to be found on the gravel. When all of the hides were transferred to the gravel a similar preference was shown for hides ($P<0.001$), but those not selecting hides were found on the mud. When an excess of animals to hides existed, the preference for hides remained ($P<0.001$) but those outside the hides also showed a marked preference for the mud ($P<0.001$). Hide sharing was very rare and in fact was only observed on three occasions, two of which related to this latter trial when there was an excess of animals.

It thus appears that hide availability is more likely to be the limiting factor in the distribution of crayfish than is the substrate, although the type of substrate may govern hide availability. The second factor examined in relation to distribution of crayfish along the Leen was water quality. This was conducted by examining two reference sources. One was data obtained from the S.T.W.A. relating to the physical and chemical parameters of water quality at eight sampling points along the length of the Leen, (see Fig. 1.1). The mean results of this data are illustrated in Figs. 3.6 a-i, and relate to the period 1980/81. The second reference source was Saleem (1980) which has data relating to the biological parameters of water quality and the change along the length of the river.

Sample site 1 of the S.T.W.A. is at Newstead Abbey and relates to the situation in the East branch of the Leen prior to its confluence with the West branch. All other sites relate to the river after the confluence, and therefore to the region in which no crayfish were found. Their positions along the river are described in Table 1.1 and illustrated in Fig. 1.1. From Figs. 3.6 a-i it may be seen that a dramatic increase is observed on moving from site 1 to site 2 and beyond, in the levels of; conductivity, suspended solids, chloride, alkalinity, hardness, and nitrogen compounds. Disolved oxygen levels and pH both fall slightly, whilst the temperature remains similar along the length of the river, although the most extreme values may be observed in the East branch at Newstead Abbey.

Considering the biological parameters of the river, it is reported that the very uppermost reaches of the East branch have

a low species diversity. This, however, increases from below the Lower Lake until at the footbridge a high degree of diversity is attained, including a number of pollution intolerant species of Plecoptera. By contrast, the polluted west branch has a very poor diversity of macro-invertebrates right up to the confluence, beyond which the fauna is intermediate in quality. A further deterioration is observed at Bestwood, possibly due to mining effluent, and this trend is continued towards the Trent as urban run-off enters the Leen within the boundaries of the City of Nottingham (Saleem, 1980).

Thus it may be seen that in biological, physical, and chemical terms, a deterioration of water quality is observed on moving downstream from the East branch of the Leen. This deterioration is noticeably apparent directly after the confluence of the two branches of the river. Comparing this with the distribution of crayfish reported above, it is apparent that they appear to be confined to the unpolluted parts of the river. Their abundance within this area, the East branch, also coincides with the change of species diversity observed, increasing from below the Lower Lake to the footbridge. It therefore seems that the crayfish population is governed by two factors. One is the water quality which limits distribution, whilst hide availability limits their density. Crayfish were observed in areas of sand/mud, but only under rocks when present, and so substrate variability itself did not apparently affect distribution.

3.3(ii) MARKFIELD QUARRY

The topography of Markfield Quarry has been described (see 1.2). Figs 1.3 and 1.4 illustrate the steep sided nature of the

quarry, showing that it has only three areas where shelving occurs, which themselves drop almost vertically to the bottom after a few metres. It is the shelving areas, however, which provide the most suitable habitat for crayfish, since it is here that many large and small rocks occur as potential hides. The steep sides of the quarry have few crevices, and the bottom is covered with very fine mud.

During October and November 1979 SCUBA was used to enable a survey of the quarry and also in order to assess the areas where crayfish were most abundant. The October dive was conducted just prior to dusk. Two teams of two divers entered the water and swam along compass bearings in the directions indicated in Fig. 1.3. Each diver counted the crayfish observed in a one metre band on his side of the (imaginery) compass line. In transect 1 only 14 crayfish were counted, whilst 44 were observed in transect 2. How this relates to population density is discussed in 4.1, whilst their distribution is of interest at this point. The lower count occurring in transect 1 is related to the fact that it was mainly across the muddy bottom whilst transect 2 covered two of the shelving areas. It was apparent that these areas had the highest densities of crayfish, particularly within the top 2-3 metres. The bottom of the quarry, however, was also found to be inhabited by crayfish, although they were less abundant. In the absence of rocks for hides, the *cladophora* present seemed to be serving this function, and holes/burrows were common in the cotton-wool like mass. It was presumed that crayfish had made these as hides.

The second dive made during November was principally to

lay a transect line, but also served to confirm the above observations on distribution and relative abundance. It also agreed with the observation that fewer crayfish were out of their hides during the day time than at dusk.

From trap placements it was not possible to assess whether any seasonal variation was occurring with regard to the depth at which crayfish could be found. The catches each month were nearly always in the third and fourth traps for both of the sets of five. Thus this was considered to be a result of where the traps happended to fall, rather than of the depth at which they occurred.

DISCUSSION

In Britain there is only one indigenous crayfish species, *A. pallipes* (Thomas and Ingle, 1971; Gledhill *et. al.*,1976), which, as has previously been mentioned, enjoys a wider ecological range than the same species in Europe (see General Introduction). The distribution of *A. pallipes* in Britain was reviewed by Thomas and Ingle (1971), and more recently this has been updated by Jay and Holdich (1981), based on information they received from water authorities, and upon personal observations. It is found that crayfish are both widely distributed and abundant, but they appear to be limited to areas with base rich, easily weathered substrata, where the water bodies have a pH of 7-9. This applies to chalk and limestone areas, and some sandstone areas which have been influenced by limestone (Jay and Holdich, 1981).

When considering crayfish throughout the world it is found that the factors which may affect distribution are often species specific (e.g. Bovbjerg, 1952; Bouchard, 1974; Terman, 1974).

In general, however, similar constraints are involved for most crayfish species, and these include temperature, the type of substrate, calcium concentrations, dissolved oxygen levels, light conditions, water level variations and the action of current, food availability and the distribution of plants, pH, and man induced factors within the environment such as pollution or channelization (Bovbjerg, 1952; Davies, 1964; Chaisemarten, 1967; Kossakowski, 1971; Abrahamsson, 1972b; Kossakowski, 1972; Westman, 1972; Cukerzis, 1974; Hobbs and Hall, 1975; Lake and Newcombe, 1975; Jay and Holdich, 1976, 1981). Certain of these factors will have a limiting effect on the overall geographical distribution of a species, whilst others will have only local effects. For example, temperature is known to limit the northerly distribution of *Astacus astacus* in Sweden (Abrahamsson, 1972a), and Finland (Westman, 1972), and that of *A. pallipes* in Britain (Brown, 1979). Cold water temperatures prevent the development of the eggs, and the growth of the juveniles, although adults may survive (e.g. Abrahamsson, 1972a), and it has been shown that at 7°C although eggs of *A. pallipes* may hatch, they do not survive (Rhodes, 1981).

Another factor having major limiting effects on distribution is the calcium concentration of the water. Chaisemarten (1967) has suggested levels of between 2-8 mgl^{-1} calcium ions as being limiting for crayfish, whilst Greenaway (1974) postulates that the threshold level is 5 mgl^{-1} for *A. pallipes* and Lilley (1979) reports that in the Wye catchment area, no crayfish occurred where the calcium concentration was less than 6.1 mgl^{-1}. Certainly it has been reported that crayfish (*A. pallipes*) have difficulty

in moulting when maintained in water from Lake Windermere, which is low in calcium (5-8 mgl^{-1}, Sutcliffe and Carrick, 1975). The calcium is required for hardening of the exoskeleton and may be obtained in ionic form, or from the food (Greenaway, 1974).

It is the elements in the environment which may affect the local distribution of crayfish which are of most relevance to this study. In the River Leen it was found that substrate proved to be a limiting factor to the distribution of crayfish only in as far as the provision of hides was concerned. The requirement for suitable retreats appears common, and in general a firm type of substrate with many larger stones is preferred (*O. virilis* : Camougis and Hichar, 1959; Bovbjerg and Stephen, 1974; *O. propinquus:* Bovbjerg, 1952; *A. pallipes:* Brown and Bowler, 1976; *A. astacus:* Niemi, 1976). This of course does not apply to the burrowing species which tend to inhabit muddy areas (e.g. *Cambarus fodiens:* Bovbjerg, 1952; *Parastacoides tasmanicus:* Lake and Newcombe, 1975; *Paranephrops sp:* Chapman and Lewis, 1976; *Procambarus clarkii*: Lowery and Mendes, 1976), though the requirement for a retreat is still apparent. This requirement is exhibited very well by an example of *A. pallipes* in Britain. Crayfish used to be found in certain parts of the River Upper Witham, but now their distribution is limited due to a vegetable processing plant, the effluent of which enters the river. It is high in calcium bicarbonate which has caused precipitation of calcium carbonate blocking the interstices between rocks, thus destroying the hides of the crayfish (Ackroyd, pers. comm.).

Tests made in the artificial river at Nottingham showed quite significantly that a preference for hides occurred. Even

when there were more crayfish than retreats, however, hide sharing proved to be very rare. *O. virilis* has been found to actively defend individual crevices in the substrate, and in laboratory experiments it was found that 96% of all hides were occupied singly. However, when a considerable excess of animals to retreats occurred, sharing was observed (Bovbjerg and Stephens, 1974). In the wild, burrows of *O. virilis* were rarely seen to be occupied by more than one animal (Hazlett *et. al.*, 1974). Such observations suggest territoriality, although no evidence of such was seen to exist for *A. pallipes* in the artificial river. For natural populations of *A. pallipes*, however, it has been observed that a foraging animal would be challenged by another as yet unemerged from its hide, if the former were to enter its 'territory' (Ingle, 1979). Since hide sharing is rare, the type of substrate therefore, is likely to limit not only distribution, but also population density. This is clearly shown for *P. leniusculus* in Lake Tahoe, California. Where many hides occur a large number of small crayfish may be found, but with few hides present, only a small number of large crayfish tend to exist (Abrahamsson and Goldman, 1970).

In Markfield Quarry, crayfish were observed to exist on both the mud of the bottom, and the rocky sides. However, in agreement with the previous findings, they were more abundant in the rocky areas where there was more provision of hides. The 'hides' observed in the *Cladophora* on the bottom were also thought to be made by crayfish, so clearly the requirement for hides remains. It is also possible, based on observations in the artificial river, that some animals may bury themselves in the mud to avoid predation during the day. In the substrate selection experiment,

the observation that those animals not selecting hides tended to prefer the mud to the gravel substrate may be explained by this argument. An alternative explanation, which in addition may also explain the preference for hides on the mud (regime (2)), could be food availability. The mud had been obtained from the River Leen, complete with its associated fauna, whilst the gravel was simply obtained from the engineering department of the University.

Another factor limiting the local distribution of crayfish then, may be food availability. The distribution of *Parastacoides tasmanicus* has been shown to be related to the distribution of the plant communities on which it feeds (Lake and Newcombe, 1975). Cukerzis (1974) also reports that distribution of *Astacus astacus* is related to food availability, and it has also been suggested that differences in the local distributions of males and females may be food related, with dominant males occupying the prime areas (Abrahamsson, 1966). In the River Leen, species diversity increased on moving from the Lower Lake to the footbridge, and similarly the population density of the crayfish also increased. This, however, may not have been due to increased food availability as much as improved biological quality of the water. On a more local scale, crayfish tended to be caught in the same areas as possible food resources, simply because the weirs and large stones accumulated organic matter, and hence also other invertebrate life. At Markfield, the greater population density of crayfish observed near the surface may perhaps be related to the distribution of food organisms, although this was not established.

For the River Leen, a second major factor reported as limiting

the local distribution of crayfish, was water quality. Pollution of water bodies has been commonly recognized in various countries as being a major cause for the destruction of crayfish populations, or for limiting their present distribution (Davies,1964; Kossakowski, 1971, 1972; Goldman, 1972; Hobbs and Hall, 1975; Erensin and Koksal, 1976; Jay and Holdich, 1981). In Britain, the distribution of *A. pallipes* is reported as being not entirely restricted to unpolluted waters (Aston, Langford, in Brown, 1979), although Davies (1964) stated that this species is very sensitive to pollution, especially heavy metals, and he attributed their local distribution in the River Stow to sewage and factory effluents. Greater tolerance is shown towards organic pollution, and limited organic enrichment may even tend to increase population densities (Hobbs and Hall, 1975). The increasing use of pesticides, however, is becoming increasingly recognized as a potential 'modern' threat to crayfish (e.g. Cukerzis, quoted in Kossakowski, 1971; Goldman, 1972; Bowler, 1979).

Aspects of the water quality such as pH, oxygen levels, and temperature which have previously been mentioned as possible factors limiting the distribution of crayfish do not alter significantly in the River Leen on moving downstream from the East Branch. The pH along the full length of the river is within the range 7-9, stated as being necessary for *A. pallipes* (Jay and Holdich, 1976). Low oxygen levels, to which this species is susceptible (Davies, 1964; Downs, 1966), do not occur, and the temperature, being chiefly a function of latitude, remains virtually unchanged along the river. A dramatic difference, however, is observed in the hardness, but it is an increase which occurs,

not a decrease, and at no point along the river do levels fall to anything like the threshold limits reported (Chaisemarten, 1967; Greenaway, 1974). Thus it must be some other aspect of water quality which is having a limiting effect on distribution.

The conductivity is a measure of the nutrient and electrolyte levels in the water. In the East branch of the Leen its value is perhaps higher than might be expected, possibly due to agricultural run-off, and also due to sewage effluent from Newstead Abbey entering the river. It used to enter the top end of Lower Lake where it would have been partly purified. However, the Lower Lake no longer exists, but due to an improved sewage treatment plant at Newstead Abbey in recent years, the problem of sewage effluent entering the Leen has been reduced (Saleem, 1980). It may, however, explain the conductivity levels. After the confluence the conductivity is seen to increase dramatically, which is due to the coal mining activities at the top end of the West branch. The resulting changes in the levels of electrolytes in the water may be detrimental to the crayfish. Certainly, chloride levels are seen to increase beyond the confluence. However, anions such as this are generally considered to be relatively harmless (Doudoroff *et. al.* 1951) and the toxic properties are attributed to the metallic cations. Saleem (1980) suggests that the extremely high levels of calcium observed may have a toxic effect on fish, and possibly therefore on crayfish also, due to a reduction in permeability and water flow across the gills. He also implicates the presence of heavy metals, and in this study it was certainly found that the levels of various metals were greater below the confluence than in the East branch (see Part II).

Another factor of the water quality which is observed to increase significantly beyond the confluence of the two branches is suspended solids. This may be due to sewage or mining effluents, and probably contributes to the observation that the substrate is far more mud and silt based below the confluence. Siltation covers hides and results in deep, soft, muddy river bottoms which may be detrimental to crayfish, although in suspension, the solids are not considered to be a problem (Hobbs and Hall, 1975).

Thus it appears that no single factor of the water quality may be stated as being the causative parameter in limiting crayfish distribution. The best explanation is that a combination of factors act together to make the river unsuitable for crayfish, which singly may not have affected distribution. These include both aspects of the water quality, and the nature of the substrate.

At Markfield Quarry pollution is not a problem, and all the water quality criteria which may be possible limiting factors fall well within the requirements for *A. pallipes* (see Table 1.3). At this site, however, differences in distribution of another nature may be observed. Due to the shallowness of the Leen all discussion has related to lateral distribution. In Markfield Quarry vertical distribution in terms of the depth at which crayfish may be found is of interest, although, of course, they remain benthic organisms. It was reported that they were most abundant in the top 0-3 metres in the shelving areas of the quarry, although they were also observed at the maximum depth of 8.5 m. This situation was true in June, September, October and November, the months when either snorkelling or diving was conducted. Trapping failed to reveal whether any seasonal changes in vertical distribution

occurred.

Considering the situation for other lake dwelling crayfish populations, a variety of distributions are reported, which include seasonal migrations. Kossakowski (1971) suggests that as a rule the depth limit for crayfish is 5 m although *A. leptodactylus* have been found at up to 30 m in the Caspian Sea. *P. leniusculus* in Lake Tahoe is reported to have the most dense populations at a depth of 10-20 m, and 90% of the population occur within the range 0-40 m. The factors limiting distribution in this case are light and wind action above 10 m, and cold temperatures below 40 m (Abrahamsson and Goldman, 1970; Goldman, *et.al.*, 1974). Seasonal migration of this population is also seen to occur. During the summer and autumn crayfish tend to occur in shallow water near the shore, whilst in the winter, as both temperatures and day length decrease, they migrate into deeper water. The suggested reason for this migration is that it is a survival strategy to avoid the bad storms of the winter (Flint, 1977).

Seasonal migrations in lakes also occur in relation to the reproductive state of the animals. *P. clarkii* moves into shallow water to reproduce and egg bearing females are found in shallow water (Lowery and Mendes, 1976). Ovigerous females of *Orconectes propinquus* also tend to occur in shallow warmer water (Capelli and Magnusson, 1974), as is also the case for *P. leniusculus* (Flint, 1977). Newly hatched juveniles of the former species tend also to remain in shallow water of less than 3 m, but will have migrated to about 10 m by the winter. Momot and Gowing (1972) report that migration of *O. virilis* to deep water occurs during the winter probably to aid maturation of the gonads. These movements are

regulated by temperature. It is this factor which is also believed to affect the migration to deeper water of *A. pallipes*. Morriarty (1971, 1972) working on a population of *A. pallipes* in White Lake, Ireland, found that with traps placed at 3 metres and above, and at 15 metres, more crayfish occurred in shallow water in the summer, but migrated to deeper water in the winter. The depth of 0-3 metres observed to contain the majority of crayfish compares favourably with the situation at Markfield Quarry.

Some of the constraints to local distribution reported above for lake dwelling crayfish do not occur for *A. pallipes* in Markfield Quarry, and hence similar migrations may not be expected to occur. Surprisingly the water temperature was not observed to vary between the surface and the bottom, and indeed, the total depth of 8.5 metres may not be sufficient to reveal any seasonal changes in distribution. It is quite feasible that this distance may be covered whilst foraging, for example. Ovigerous females were rarely encountered and 0+ juveniles were never caught. It was therefore not possible to state at which depth they tended to occur.

Regarding the possibility of a home range, there have been no reports of such for lake dwelling crayfish, and movements are generally described as being totally random (Camougis and Hichar, 1959; Abrahamsson, 1966). In both lakes and rivers, greater movement is often attributed to males than females, often because females may be ovigerous and less active (Merkle, 1969; Cukerzis, 1974; Flint, 1977). The movement of crayfish in rivers is easier to monitor than in lakes, and it is perhaps for this reason that evidence for a home range has been shown to exist in this environment.

Black (1963) concluded that the home range of *Procambarus* sp. was approximately 30 m. Merkle (1969) using a mark and recapture technique found that most recaptures occurred within 30 m of the original site for *Orconectes juvenalis*. This latter technique has also been employed on a population of *O. virilis* over a 12 month period. Only animals recaptured at least four times were used, and it was found that considerable variability of movement existed. Some crayfish did not move at all, whilst others moved up to 308 m. The average capture-capture movement however, was 33 m, and no significant differences were observed between males and females (Hazlett, *et. al.*, 1974). A more precise survey of home range was conducted by tagging crayfish (*O. juvenalis*) with a radioactive cobalt source so that individual movements could be monitored. It was found that home ranges varied from 9.4 - 60.4 m^2 in area, and 9.4 - 47 m in length, the average being 32.7 m^2 in area and 23 m long. The differences observed could not be related to sex or size, although it was observed that the tendancy of ovigerous females to move was reduced (Merkle, 1969).

Another fact discovered by the latter author was that during flooding crayfish were not washed downstream as had been suggested by Momot (1966), who claimed that upstream migrations took place to repopulate streams. Similarly, Hynes (1972) suggests that upstream migrations of various invertebrates, including Crustacea, occur during the summer months. Evidence to support this assertion is apparent for *Astacus klamathensis* in Spring Creek, Oregon. Downstream migrations were observed to occur in April and May, and consisted mostly of females. Upstream migrations occurred

from September to November (Henry, 1951). The tendancy for *A. pallipes* to move upstream when released has been reported by this author (see 2.1), and by Brown (1979). The possibility that they may be washed downstream has also existed with intermittent changes in the flow of water caused by factors such as the emptying of Lower Lake and cleaning of Newstead Abbey lakes (Scruby, pers. comm.). This, however, is considered unlikely, and from the observations of Merkle (1969), probably would not have occurred.

Thus it may be seen that the evidence for and against the existence of a home range is conflicting. For *A. pallipes* in a Northumberland aqueduct no evidence of home range was found to exist, and the distance that crayfish moved was seen to increase with time from release. Movements were random in all directions (Brown, 1979). In contrast, the results presented here indicate that a home range probably does exist for *A. pallipes* in the River Leen. The method employed was a mark and recapture programme over a period of two years, similar to those of Merkle (1967) and Hazlet, *et.al.*,(1974). It was not possible to restrict analysis to multiple recaptures since this did not occur sufficiently frequently, hence each individual recapture was considered. It was found that most crayfish are recaptured at the original site, and that of those moving, more tended to move downstream than upstream. Distances moved tended to be quite small, the average being 28.8 m, which compares favourably with the home ranges reported above for other species. No differences between the sexes were observed.

It is of course possible that many of the crayfish moved such large distances that they migrated out of the study area.

If this were the case, however, one should have expected to find more marked animals when the whole of the East branch was surveyed on the two occasions discussed. Also animals marked and released outside the study area were never captured in that area. Also of a contradictory nature is the fact that by the end of the study 1,271 animals had been marked, and the maximum potential catchable population for the study area was predicted at being around 600 (see 4.1). Why then were not all of the animals captured towards the end of the two year study period marked? Some of this discrepancy may be explained by the fact that the largest proportion of the catch is represented by the youngest year classes, and the new recruitment will bear no marks. The larger animals carrying marks may be the subject of mortalities. Alternatively, the calculated population sizes may be underestimates. Fig. 3.7 shows that in fact the percentage of the catch bearing marks did increase towards the end of the two years. This would not have occurred if considerable migration out of the study area was occurring.

Heavy metals were not found in the East branch of the Leen, but they do occur after the confluence (see Part II). Analysis of the tissues of the crayfish from the study area reveals small amounts of certain metals and so this might also tend to indicate that greater movement occurs than is suggested above. The movement, however, need not necessarily be of the crayfish themselves, but could be of their food organisms. Concentrations of the metals would then occur within crayfish tissues, and thus it is impossible on these grounds to make a definitive statement about their movements.

In conclusion, it appears that certainly in the River Leen, some evidence for the existence of a home range for *A. pallipes* does exist. Local distribution is affected by substrate and hide availability, and this is observed in both of the populations studied. The population density is also governed by these factors, as has been demonstrated for *A. pallipes* in France where it was found that the provision of hides was of the greatest importance for maintaining high population densities (Daguerre de Hureax and Roqueplo, 1981). Movement outside of the normal home range therefore may occur to prevent overcrowding, and it has been shown in a laboratory study that a direct relationship exists between population density and the rate of movement of crayfish (Bovbjerg, 1952). However, it is also apparent from this study that movement and distribution may be limited by other factors prevailing locally, such as a change in the water quality. This is advantageous for some species which may be more pollution tolerant, and thus may occupy a vacant niche left by pollution intolerant species. The spread of *O. limnosus* over a wide area of Germany has been possible since it is relatively tolerant of pollution (Schweng, 1972). In general, however, poor water quality is a limiting factor to the distribution of crayfish. In recent years the effects of pollution have been recognized and dealt with more positively than in the past. For example, the rivers of the Trent system have been improved by increasing controls over effluents, and it is envisaged that once polluted rivers such as the Tame will be restored sufficiently that they may be able to support good fisheries again (Woodward, 1980). Restoration of this nature will obviously enable the reestablishment

of crayfish populations which in the past may have been decimated by pollution, such as in the Trent.

TABLE 3.5 POPULATION ESTIMATES ALONG THE EAST BRANCH OF THE LEEN USING PETERSON ESTIMATE, WEIGHTED MEAN (≡ LINCOLN INDEX)

LOCATION	1980					1981				
	N	RECAPt.	MARKED	$\hat{N}$	±SE	N	RECAPt.	MARKED	$\hat{N}$	±SE
LOWER LAKE TO GAS MAIN	* 12	17	2	72	33	12(0)	20(86)	1	126	69
GAS MAIN TO FPRD						19(36)	5(2)	-	-	-
FORD TO ISLAND	46	70	1	1633	929	29(1)	33(3)	2	329	157
ISLAND TO WEIRS	47	37	1	893	501	33(0)	36(1)	-	-	-
WEIRS (POPn. STUDY AREA)	56	35	2	672	321	81(60)	33(21)	12	212	44
BELOW FOOT-BRIDGE	10	33	5	57	6	19(11)	*11(10)	2	168	73
FOOTBRIDGE → WEIRS(S)	28	26	2	252	118	23(14)				
BELOW CONFLUENCE NEAR BRIDGE	3	-	-	-	-	-	-	-	-	-
TOTALS	203	218	13	3578	1909	216(122)	138(123)	17	>835	>343
POPn. EST.BASED ON TOTALS				3176	793				1668	357

NOTES: N = No. first caught.
$\hat{N}$ = Simple Petersen population estimate, ± Standard error.
(x) = No.of 0+ juveniles caught, excluded from population estimates.
* = These areas were accidentally pooled in the years shown.
S.E. = Standard error.

TABLE 3.6 A SUMMARY OF SUBSTRATE PREFERENCE EXPERIMENTS CONDUCTED IN THE ARTIFICIAL RIVER AT NOTTINGHAM UNIVERSITY

REGIME ADOPTED	NUMBER OF HIDES USED	NUMBER OF CRAYFISH	MEAN PROPORTION OF CRAYFISH IN HIDES (%) ±S.E.			MEAN PROPORTION OF CRAYFISH NOT IN HIDES (%) ±S.E.			% OCCURRENCE OF CRAYFISH OCCUPYING SAME HIDE > TWICE	% INCIDENCE OF HIDE SHARING
			HIDES ON MUD	TOTAL IN HIDES	HIDES ON GRAVEL	CRAYFISH ON MUD	TOTAL NOT IN HIDES	CRAYFISH ON GRAVEL		
(1) NO HIDES	0	10	-	-		33.3 ±14.4	66.7 ±14.4		-	-
(2) HIDES:- 50% ON MUD 50% ON GRAVEL	10	10	50.5 ±8.5	92.0 ±13.8	41.5 ±5.3	7.2 ±7.5	8.1 ±10.3	0.9 ±2.76	60	0.67
(3) HIDES:- 100% ON MUD	10	10	87.7 ±16.9	87.7 ±16.9	-	-	12.3 ±16.9	12.3 ±16.9	57	0
(4) HIDES:- 100% ON GRAVEL	10	10	-	91.1 ±13.5	91.1 ±13.5	8.9 ±13.5	8.9 ±13.5	-	0	0
(5) HIDES:- 100% ON MUD	10	16	81.2 ±3.6	81.2 ±3.6	-	15.3 ±3.1	18.8 ±7.4	3.5 ±4.3	23	2.20
(6) HIDES:- 50% ON MUD 50% ON GRAVEL	20	16	51.9 ±3.8	100	48.1 ±3.8	-	0	-	0	0

TABLE 3.7 ANALYSIS OF SUBSTRATE PREFERENCE EXPERIMENTS BY THE t-TEST

REGIME	COMPARISON	t	df	P
1	NUMBER ON MUD/GRAVEL	2.8407	4	<0.05 > 0.025
2	NUMBER IN HIDES/NOT IN HIDES	14.6370	15	<0.001
2	% IN HIDES; ON MUD/GRAVEL	2.6954	13	<0.025 > 0.01
2	% NOT IN HIDES; ON MUD/GRAVEL	2.3649	10	<0.05 > 0.025
3	% IN HIDES/NOT IN HIDES	7.0543	8	<0.001
4	% IN HIDES/NOT IN HIDES	8.6110	6	<0.001
5	% IN HIDES/NOT IN HIDES	20.0620	9	<0.001
5	% NOT IN HIDES; ON MUD/GRAVEL	5.8895	11	<0.001
6	% IN HIDES; ON MUD/GRAVEL	1.4142	6	>0.2

FIG. 3.5

The upper reaches of the River Leen showing the relative abundance of crayfish on a scale of 0-4. Areas A-F relate to changing substrate conditions and are discussed in the text (see 3.3(i)).

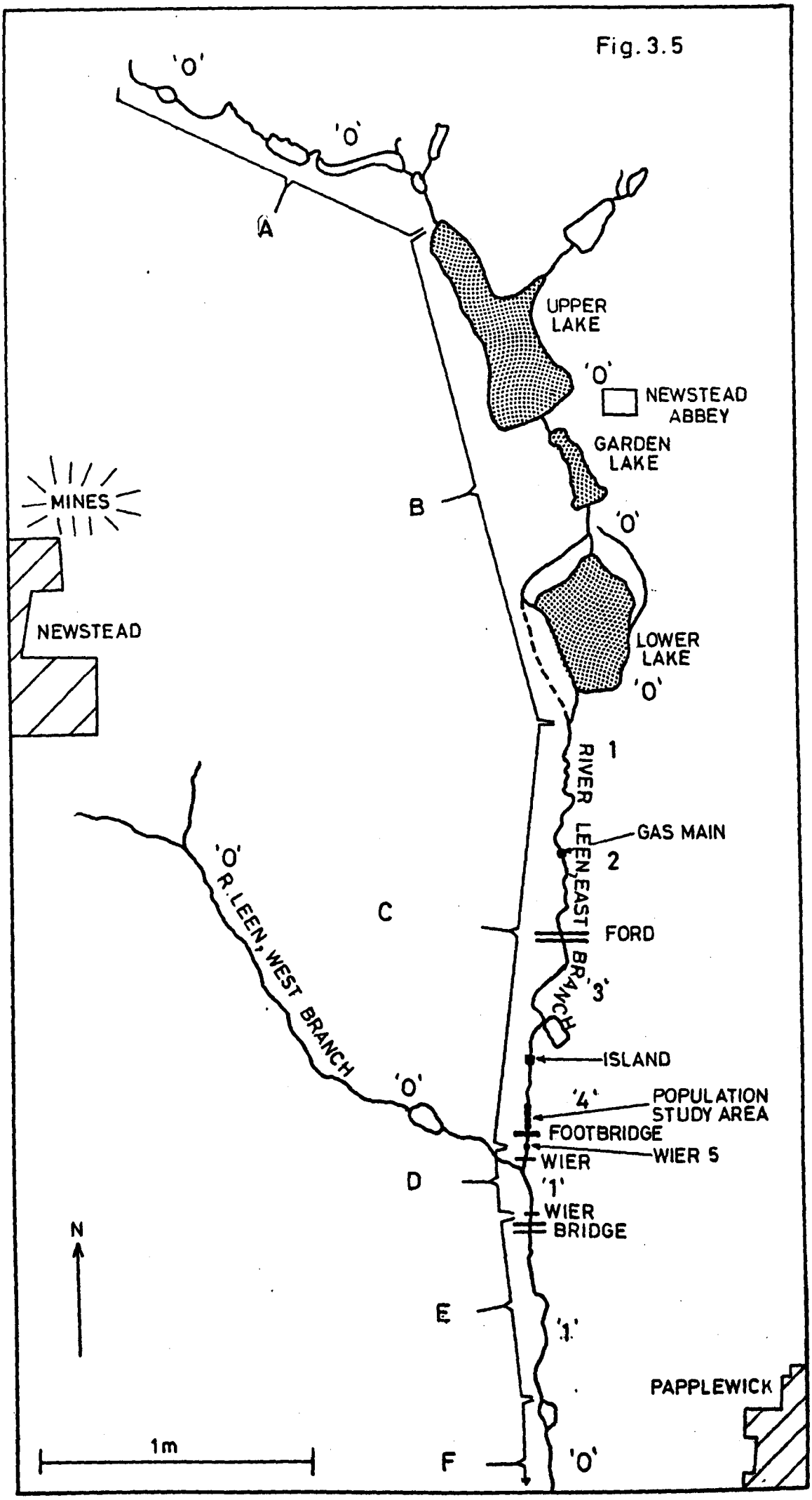


Fig. 3.5
'O'
'O'
A
UPPER LAKE
'O'
NEWSTEAD ABBEY
GARDEN LAKE
MINES
B
'O'
NEWSTEAD
LOWER LAKE
'O'
1
RIVER LEEN, EAST BRANCH
GAS MAIN
2
'O' R. LEEN, WEST BRANCH
C
FORD
3
ISLAND
'O'
4
POPULATION STUDY AREA
FOOTBRIDGE
WIER
WIER 5
D
'1'
WIER
BRIDGE
N
E
'1'
PAPPLEWICK
1m
F
'O'

FIG. 3.6(a) - (i)

These figures illustrate the physical and chemical characteristics along the length of the River Leen as represented by the averaged results for the 1980/81 period of S.T.W.A. data. The sampling site numbers 1-8 are the S.T.W.A. sampling sites illustrated in Fig. 1.1 from Newstead Abbey (1) to the confluence of the Leen and Trent (8). The distance between each sampling site is not represented to scale, and the averaged values given will not show whether any sudden changes occurred due to a flush of pollution for example. The population study area is most closely represented by site number 1.

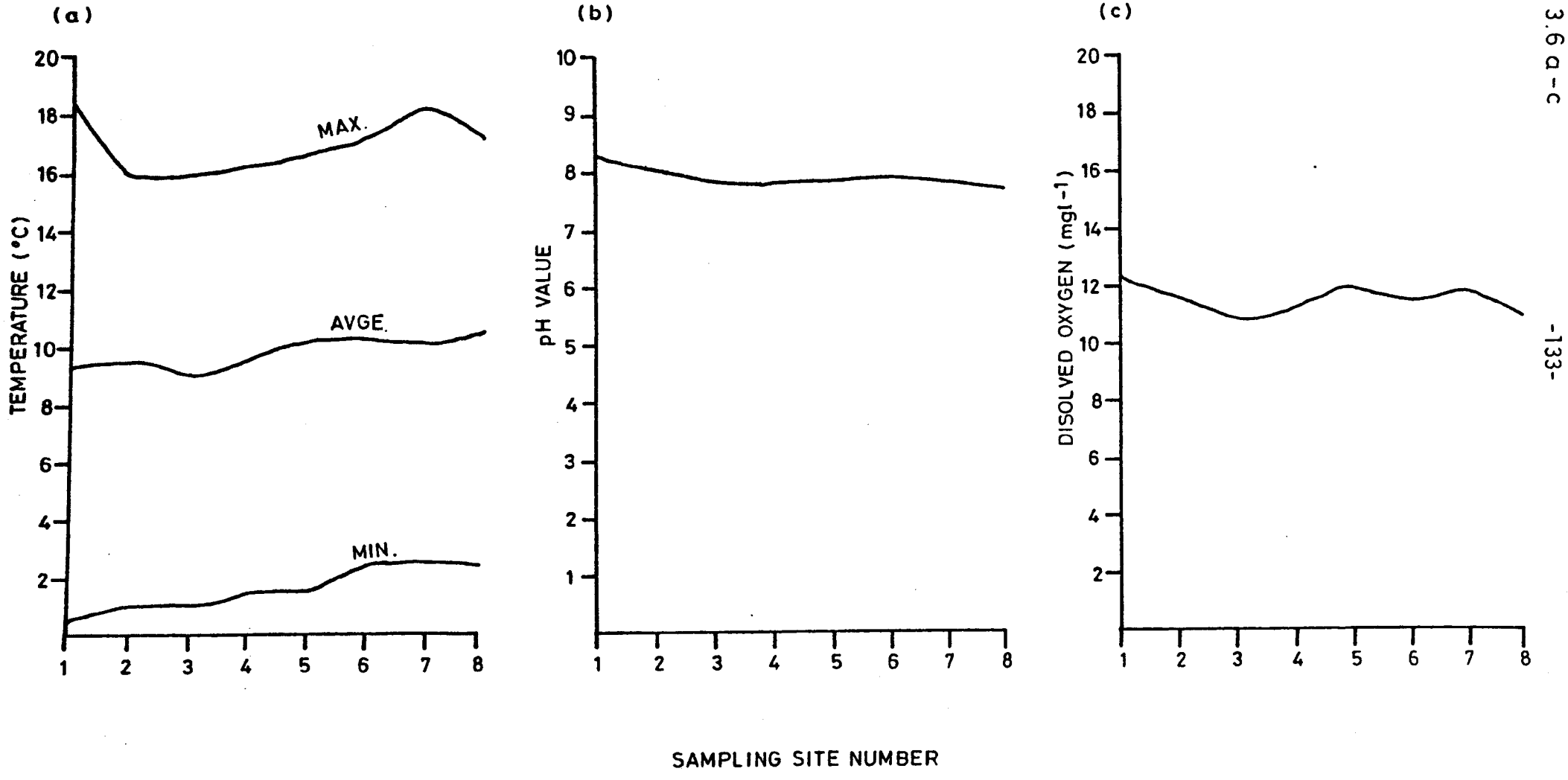

(a)
(b)
(c)
TEMPERATURE (°C)
MAX.
AVGE.
MIN.
pH VALUE
DISOLVED OXYGEN (mgl⁻¹)
SAMPLING SITE NUMBER

Fig. 3.6 a-c

Fig. 3.6 d–f

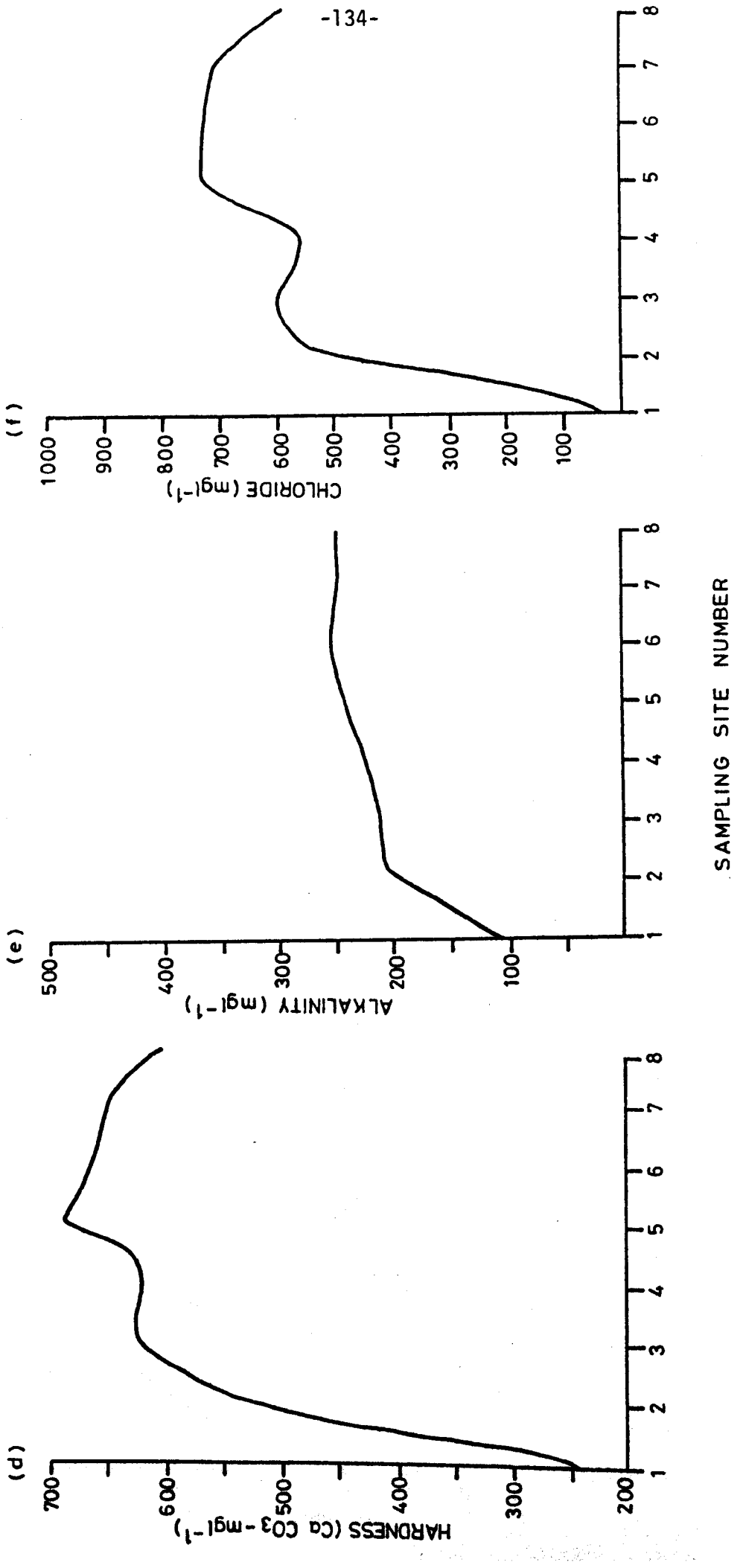

(d)
HARDNESS (Ca CO3 - mgl-1)
700
600
500
400
300
200
(e)
ALKALINITY (mgl-1)
500
400
300
200
100
(f)
CHLORIDE (mgl-1)
1000
900
800
700
600
500
400
300
200
100
1 2 3 4 5 6 7 8
SAMPLING SITE NUMBER

Fig. 3.6 g-i

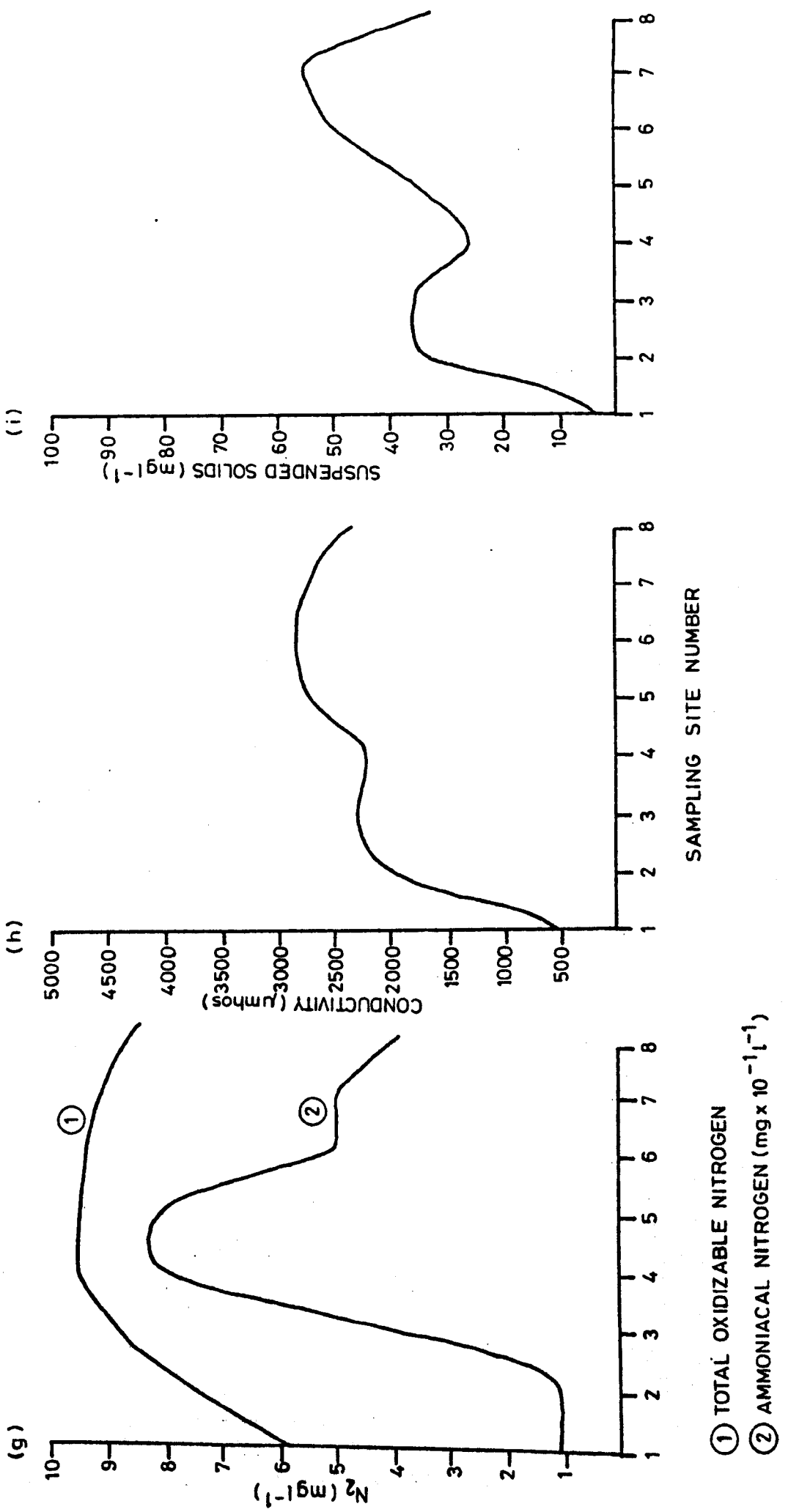

(g)
N_2 (mgl^{-1})
(h)
CONDUCTIVITY (μmhos)
(i)
SUSPENDED SOLIDS (mgl^{-1})
SAMPLING SITE NUMBER
① TOTAL OXIDIZABLE NITROGEN
② AMMONIACAL NITROGEN (mg x 10^{-1}l^{-1})

FIG. 3.7 TO SHOW THE PROPORTION OF LEEN ANIMALS BEARING MARKS WITHIN EACH MONTHLY CATCH

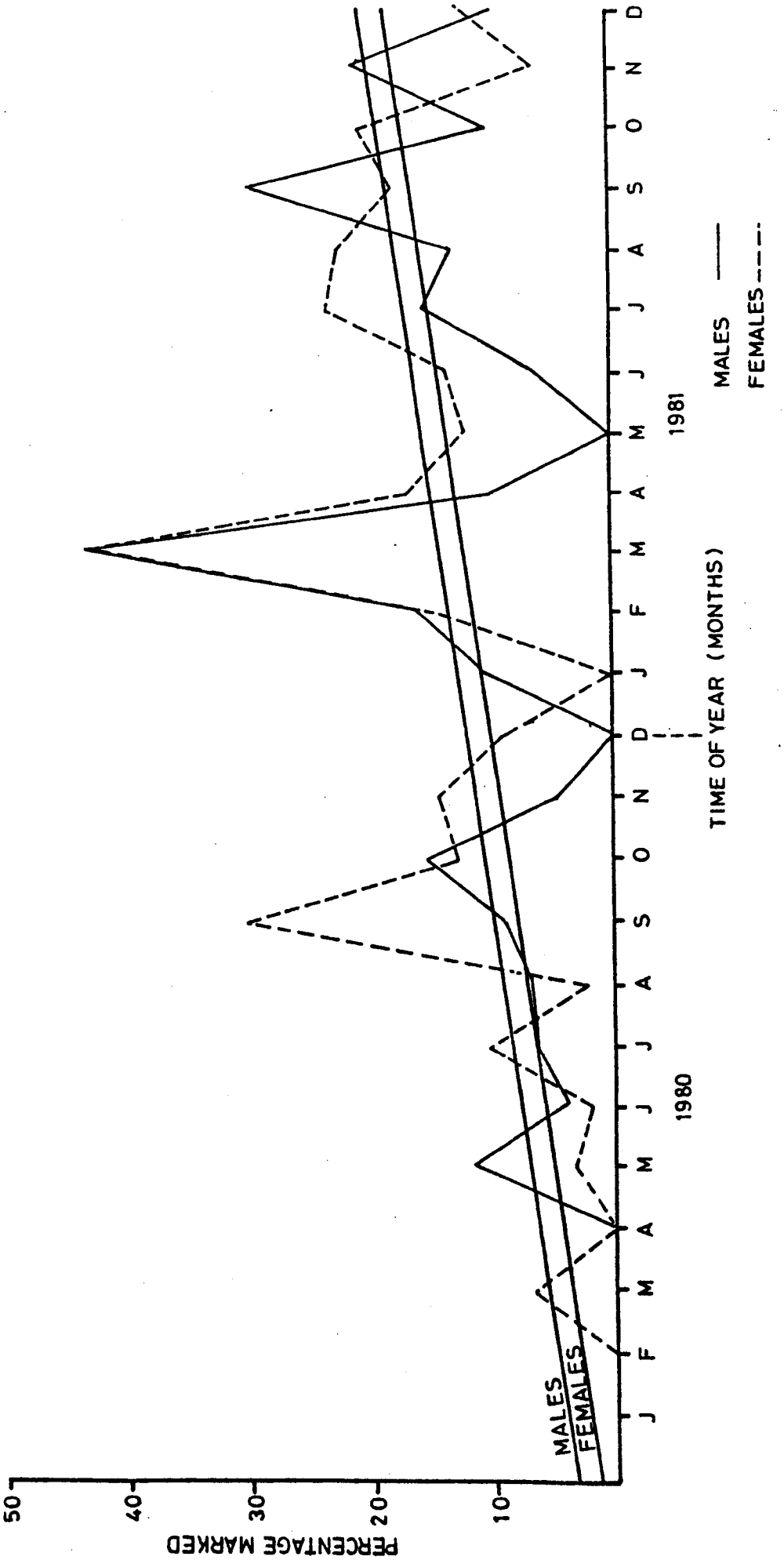


PLATE 3.1

This photograph illustrates the arrangement of containers used to house the ovigerous females of the Nanpantan stock. They were arranged in the outside holding tanks, suspended on wire mesh. Each container had a small plastic tube in it for a hide, and each had its own individual water supply.

PLATE 3.2

This photograph illustrates the artificial river, constructed to enable substrate preference experiments to be conducted.

PLATE 3.1

PLATE 3.2

CHAPTER 4

POPULATION STUDIES

4.1(i) INTRODUCTION

There are several methods of estimating population size, all of varying accuracy and reliability (e.g. see Emmel, 1976). An absolute count of the population would obviously be the most accurate, but this is seldom possible except with small closed populations. Area-estimates such as strip censuses and quadrat sampling are useful guides, but often are not as accurate as required, particularly for mobile animals which may escape from the quadrat or strip being surveyed. The strip census involves counting all the animals along a transect line, but they should be randomly distributed, and all should be equally visible otherwise inaccurate estimates of population size will result. The quadrat method is best for immobile animals, or for plants, and looks at the number of organisms within an enclosed unit area, which is then multiplied up to give an estimate of the total population. Again the assumption is that the population will be evenly and randomly distributed. This method has in fact been used for crayfish, where, using SCUBA, large containers were placed over certain areas, and all the animals inside were counted (Abrahamsson and Goldman, 1970; Flint, 1975).

Of a more complex nature are capture per unit effort models of the population, which consider several catches over a short period of time (e.g. see Emmel, 1976). A declining catch is observed which, plotted against the total catch will give an estimate of the total population when the catch per unit effort is

extrapolated to zero. However, this requires that all the population is of equal catchability over all of the catches. This generally is not the case, and so this method too is limited in its application.

Mark and recapture methods of population estimation are generally considered to be the best census method. In addition to yielding information on the population size, these methods may also provide other valuable details of the dynamics of the population such as movement, growth, sex ratio, age structure, survival rates, and gain rates. The basic theory behind these methods is that a sample of the population is caught, marked and released. On a subsequent sampling occasion some of the catch will bear marks, and the ratio of the recaptured marked animals to the unmarked animals, should in theory be the same as the ratio of the total number of marked animals to the total population. The most simple population model based on this theory was devised by Petersen (1896). This method was also developed by Lincoln (1930) so is often referred to as the Lincoln Index. Since then several more complex models have been devised which are less restrictive and may be employed with either 'open' or 'closed' populations (see Seber, 1973; Begon, 1979).

All of the mark and recapture methods make various assumptions which need to be met for the model to be strictly valid. These are:

(i) All marks must be permanent for the period of the investigation.

(ii) Marking must not affect subsequent catchability or survival.

(iii) Total mixing of the marked and unmarked population must occur.

(iv) The sampling periods should be short in relation to the total time between sampling.

(v) All individuals in the population must have an equal probability of being caught, regardless of age, sex, size, or physiological condition. Also the number of each sub-population sampled must be equally proportional to the total numbers of that subpopulation.

(vi) All individuals in the population must have an equal chance of survival.

The above six requirements apply to all models of population estimation. Single census estimates of the Lincoln Index type make the further assumption;

(vii) The population is closed between sampling occasions, and no births or immigration, or no deaths or emigration will occur.

The models which provide estimates for open populations require several mark-recapture occasions. Hence (ii) above is of added importance since it is essential that no learning is involved which may affect subsequent recapture. There are three such models commonly employed, which each have their own inherent assumptions. The Fisher-Ford (1947) model assumes that survival is constant and independent of age. It is the best method when data are sparse and these conditions may be met. The Jolly (1965) model only assumes that survival is independent of age, but requires more data, whilst the Manly Parr (1968) model requires neither of these assumptions, but even more data is needed. The choice of exactly which model to use is dependent upon each individual situation, and there are no hard and fast rules. In general,

however, the method of Jolly is thought to provide the best estimate of population size, so long as the sampling intensity is about 10% of the total population (Southwood, 1966; Emmel, 1976). Indeed, it has been said, "The advantages of this (Jolly) method are obvious. The difficulty is in deciding when not to use it" (Begon, 1979). The method, in addition to providing an estimate of the population size, also allows calculation of survival rates and gains to the population and, being a stochastic model, is more akin to reality than some previous deterministic models (Begon, 1979). In addition, the model is highly flexible and allows for differences between the initial catch size and the number of marked and released animals which may arise from handling mortalities.

Choosing the particular model is not the only problem. One must consider also its accuracy. Standard errors may be calculated, but these themselves may be questionable, and tend to be highly correlated to the population estimate, such that underestimates appear more accurate, and overestimates less accurate than they really are (Manly, 1971; Roff, 1973). Computer simulations give estimates of the sampling intensities necessary to achieve results of varying accuracy. Robson and Reiger (1964) considered the sampling intensities required for different accuracies and different sized populations using the Petersen estimate, and Bishop and Sheppard (1973) compared the Jolly and Fisher and Ford estimates, confirming that sampling intensity should be greater than 10% as stated above. For the Petersen estimate sampling intensities of 55%, 28%, and 15% are required for accuracies of 0.1, 0.25, and 0.5 respectively, and for Jolly's model these intensities

should be 40-50%, 25-30% and 15% (Roff, 1973). As a guide to the accuracy required of population estimates for different purposes, Robson and Reiger (1964) suggest the following; 0.5 for preliminary studies where only a rough idea of population size is required; 0.25 for more accurate population management work; and 0.1 for accurate work on the dynamics of a population. The dynamics such as survival and gain are themselves liable to error, and often survival rates are less reliable than the population estimates (Bishop and Sheppard, 1973).

Another problem of population estimation arises when the population is not discrete, and its limits are not easy to recognise (Begon, 1979). In these cases it is usually necessary to artificially define the borders of the 'population' to be studied. This is justified when they may be well defined by some particular feature, otherwise the population estimate becomes open to even more inaccuracy.

From all of the problems associated with estimating population size one may ask, "Is it worth it?". Clearly it is better to have some idea of the size of a population than none at all, and so the answer must be yes. The problems tend to be inherent in the fact that a true population is extremely complex, having a variety of influences acting upon it at any one time, whilst compared to this, even the most complicated mathematical model still falls short of the real situation. It is in attempting to meet all of the criteria of the models that difficulties may arise, and often they may not all be met. This being the case, any interpretation of the results based on a mathematical model must take into consideration these shortcomings and limitations,

and must be viewed in the light of all the factors discussed above.

4.1(ii) METHODS

A mark-recapture programme was not conducted at Markfield Quarry (see 1.2) and hence the estimates of population size are highly subjective, being based upon observation whilst snorkelling. A permanent transect proved not to be feasible (see 1.2) although transects were counted on one occasion. Two teams of two people using SCUBA swam along compass bearings across the width of the quarry in the directions indicated in Fig. 1.3. Each diver counted all of the crayfish in a 1 m band on his side of the 'line'. Hence from this, and from snorkelling observations, crayfish density estimates per unit area are possible, which may then be multiplied up to the full size of the quarry.

In the River Leen it was possible to conduct a mark and recapture programme. It was envisaged that each monthly sample should add to a Jolly (1965) trellis enabling population estimates over the full two year period. The full method is described elsewhere (see Jolly, 1965; Seber, 1973; Emmel, 1976; Begon, 1979), but briefly it involves marking all animals caught with a date specific mark. On subsequent sampling occasions any marked animal is recorded, then remarked with a new date specific mark. When recaptured it is only the most recent mark which is considered, and all previous marks are ignored. The data from subsequent sampling occasions is used to construct a trellis (see Table 4.1) from which the data required for the population estimate is derived. In this study individual specific marks were employed, and so it was easily possible to find the dates of original capture,

and previous recaptures, so it was considered unnecessary to have separate date specific marks for purposes of population estimation. It must also be noted that only animals of 10 mm (C.L.) and above were marked, so estimates of population size refer only to this part of the population with the exclusion of early 0+ juveniles.

The formulae used in the Jolly model are:

$$\hat{M}_i = m_i + \frac{z_i\, r_i}{y_i}$$

$$\hat{N}_i = \frac{\hat{M}_i\,(n_i + 1)}{(m_i + 1)}$$

$$SE\hat{N}_i = \sqrt{\hat{N}_i(\hat{N}_i - n_i)\left[\frac{\hat{M}_i - m_i + r_i}{\hat{M}_{i+1}}\left(\frac{1}{y_i} - \frac{1}{r_i}\right) + \frac{1}{m_i} - \frac{1}{n_i}\right]}$$

$$\hat{\phi}_i = \frac{\hat{M}_{i+1}}{\hat{M}_i - m_i + r_i}$$

$$\hat{B}_i = \hat{N}_{i+1} - \phi_i \hat{N}_i$$

where;

n_i = the number of individuals caught on day i.

M_i = the number of marked individuals caught on day i.

r_i = the number of marked individuals released on day i.

y_i = the number of individuals marked and released on day i and subsequently recaptured.

z_i = the number of individuals marked before day i, not caught on day i, but caught again subsequently.

$\hat{M}_i$ = the total number of marks at risk on day i.

$\hat{N}_i$ = the total population on day i.

$\hat{\phi}_i$ = the proportion of the day i population surviving to day i + 1.

$\hat{\beta}_i$ = the number of additions to the population between day i and day i + 1.

The method of Jolly (1965) is particularly appropriate to the River Leen situation since it allows for the population to be open, and therefore immigration and emigration may occur (immigration and recruitment, and emigration and death are synonymous for the purposes of the model). Undoubtably these factors occur in the Leen, particularly over a period of two years. However, it has already been stated that this model is most accurate when the sampling intensity is at least 10% of the total population. In view of the relatively small sample sizes achieved it was decided to supplement the monthly sampling programme with additional catches each quarter, enabling other methods of population estimation to be employed.

In certain situations it is feasible to treat an open population as though it were closed (Blower *et.al.*, 1981). In such a situation, the sampling occasions must follow each other sufficiently closely that immigration and emigration are negligible, or do not occur. This being the case, it is then possible to conduct a Petersen/ Lincoln index estimate over a single recapture period. In fact, the quarterly programme devised involved three sampling occasions. Day 1 (a Monday) was the first occasion, when animals were counted, marked, and released. Day 2 (a Wednesday), two days later, was felt to be sufficiently close to day 1 to justify the requirements for a closed population, but sufficiently long after day 1 to

allow complete mixing of the marked and unmarked animals. Day 3 (a Friday) was similarly two days after the previous occasion.

Following this programme, it was considered that the maximum amount of information could be achieved without the requirement for an excessive sampling programme which would have been both time consuming, and may also have resulted in permanent upset to the habitat, thus artificially affecting crayfish numbers. The programme enabled estimation of the population size by;

(1) The Petersen method (see also Lincoln (1930))

(2) The weighted mean (see Begon, 1979)

(3) The triple catch method (see Begon, 1979)

In addition, the triple catch method enables the estimation of gain rates and survival rates, and allows for immigration and emigration, so is relatively sophisticated for a small number of sampling occasions. In effect, it is a special case of Jolly's model. The weighted mean is subject to the same limitations as the Petersen estimate, but instead of using only one sampling occasion, it uses information gained over several, and thus provides a more accurate estimate of population size.

The formulae for these estimates are;

(1) Petersen estimate, (with Bailey's (1951, 1952, not seen) correction for bias)

$$\hat{N} = \frac{r(n + 1)}{(m + 1)}$$

$$SE\hat{N} = \sqrt{\frac{r^2(n + 1)(n - m)}{(m + 1)^2(m + 2)}}$$

(2) The weighted mean

$$\hat{N} = \frac{\Sigma M_i \, n_i}{(\Sigma m_i)+1}$$

$$SE\hat{N} = \hat{N}\sqrt{\frac{1}{\Sigma m_i+1} + \frac{2}{(\Sigma m_i+1)^2} + \frac{6}{(\Sigma m_i+1)^3}}$$

(3) The triple catch method - for the population size on day 2.

$$\hat{M}_{2,1} = \frac{m_{3,1}\,(r_2 + 1)}{(m_{3,2} + 1)} + m_{2,1}$$

$$\hat{N}_2 = \frac{(n_2 + 1)\,\hat{M}_{2,1}}{(m_{2,1} + 1)}$$

$$SE\hat{N}_2 = \sqrt{\hat{N}_2(\hat{N}_2 - n_2\left[\frac{\hat{M}_{2,1} - m_{2,1}}{\hat{M}_{2,1}} + r_2\,(\frac{1}{m_{3,2}} - \frac{1}{r_2}) + \frac{1}{m_{2,1}} - \frac{1}{n_2}\right]}$$

$$\hat{\phi}1 = \frac{M_{2,1}}{r_1}$$

$$\hat{\beta} = 1 - \left[\frac{(m_{3,1} + 1)\,n_2}{(n_3 + 1)\,m_{2,1}}\right]$$

The notification for these formulae are the same as above, with the addition of $\hat{b}_2$, which is the gain rate on day 2 itself. The two suffixes to some of the parameters refer to day i, the sampling occasion, and day j, the original marking occasion. Data from the quarterly sampling programme has also been included in the compilation of the Jolly trellis (Table 4.1).

Thus four different estimates of population size were possible, all of which were calculated and are presented below. The coefficient

of variation ($SE\hat{N}_i / \hat{N}_i$) was also calculated to gain some idea of the validity of the estimate. The smaller this ratio, the greater the validity. Indeed, Roff (1973), has suggested that this value should not exceed 0.05 for the population estimate to have any meaning, but such accuracy is rare, and certainly was not encountered by this author. In this study it was arbitrarily decided that if the coefficient of variation was less than 0.5, then further interpretation of the results was valid, but that above 0.5 the estimates were highly questionable and that above 0.75 the estimate should be discarded completely. Also calculated was the ratio between catch size (n_i) and the total estimated population ($\hat{N}_i$). This gives an idea of the sampling intensity, which should be greater than 0.1 if the Jolly estimate is to be considered accurate (see introduction). In addition, using the results obtained by Roff (1973), it was possible to gain an approximate idea of the accuracy of the Petersen estimates.

4.1(iii) RESULTS

MARKFIELD QUARRY

It was possible to gain an idea of the population size at Markfield Quarry from a strip census conducted just prior to dusk in November 1979, and from the snorkelling expeditions referred to previously (see 2.1(iii)). The lengths of the transects were 88 m (Transect (2), Fig. 1.3) and approximately 100 m (Transect (1), Fig. 1.3). The former transect was measured accurately when a weighted rope was laid across the quarry in an attempt to create a permanent transect line. The topography covered by both transects has been described previously (see 3.3). Along transect 1 only 14 crayfish were observed, and 44 along transect 2. These give

population densities of 0.07 m^{-2} and 0.25 m^{-2}, assuming that all the crayfish were seen and counted. In fact, it is likely that these are gross underestimates, since being just prior to dusk, the majority of the crayfish were probably unemerged. If one then assumes that only 10-15% of the population were observed, then these densities become 0.5-0.7 m^{-2} and 1.8-2.5 m^{-2} respectively. The differences between the two transects result from the fact that the majority of transect (1) lies across the mud bottom of the quarry, whilst transect (2) encompasses two of the shelving areas in which the greatest abundance of hides occurs (see 3.3). This is an important point since the values obtained for population density using the strip census method tend to average out any spatial differences which may be observed, but which are of prime importance in gaining an accurate assessment of the total population.

The second method employed at Markfield Quarry considered the number of crayfish caught whilst snorkelling (during September 1980, June and October, 1981, and June 1982 see 2.2). The snorkel collections were always conducted at dusk when the maximum number of crayfish may be caught due to their nocturnal behaviour (see Ingle, 1979). Area A (Fig. 1.3), a shelving part of the quarry, was the study site. Sampling was conducted over an area of approximately 20 m by 5 m, i.e. 100 m^2, and the respective monthly catches were 96, 72, 34, and 83 crayfish. Assuming that the sampling intensity was between 10 and 15% of the total population in that area, which is not unreasonable based on examination of the results for the River Leen (see below), the population densities would be 6.4-9.6 m^{-2} in September 1980, 4.8-7.2 m^{-2} in June 1981, 2.3-3.4 m^{-2} in October, 1981, and 5.5-8.3 m^{-2} in June 1982.

The variation in density observed is a function of seasonal temperature differences, resulting in variable catchability (see 4.2). Similar densities, however, are seen to exist for all except October, which was the coldest month. The mean population size for the study area, based on the months excluding October, is thus 556 ±80 crayfish to 837 ±120 (i.e. 6-8m^{-2}). Having obtained an idea of the population density it is possible to estimate very roughly the total population in the quarry. As described in 1.2, Markfield Quarry is pear shaped, 88 m wide, and approximately 100 m long. The total surface area then is almost that of two circles of 88 m and 12 m diameter, i.e. 6,195 m^2. The area of the bottom of the Quarry will be slightly larger than this, say 6,250 m^2, due to the fact that the sides slope towards the bottom in some areas. Now, the density of crayfish reported whilst snorkelling relates only to the surface 0-5 metres of one of the shelving areas known to support a higher population of crayfish, whilst most of the quarry is muddy and contains fewer crayfish. Consequently, for purposes of estimating the total population of the quarry, it is better to employ the density values obtained during the strip census, which in effect average out population density variations across the variety of substrates. From these estimates, the minimum population size would be (0.5 x 6,250) 3,125, and the maximum (2.5 x 6,250) 15,265 crayfish. The upper estimate is probably the more accurate due to the high densities observed (6-8 m^{-2}) in the shelving areas where the abundance of hides creates conditions favourable to support larger populations.

RIVER LEEN

All of the captures and recaptures made over the two year study period at the River Leen are represented in the trellis diagram (Table 4.1) which is used in the calculations of Jolly's method. The results are expressed in Table 4.2. Table 4.3 shows the data used for analysis of the population size on a quarterly basis, and includes estimates achieved with the three models described previously (see Methods). A summary of the estimates from Jolly's model are also included in Table 4.3 for comparison. The Peterson estimate and Triple Catch method give an estimate of the population size on day 2, the weighted Mean is the average population size over the three days, and the Jolly model provides estimates for day 1 and 2 (Day 3 was not included in the trellis diagram, since no animals were marked and released).

Considering first the results derived from the model of Jolly (1965), presented in Table 4.2, it is apparent from the values of the coefficient of variation ($SE\hat{N}_i/\hat{N}_i$) that on no occasion do the results approach what Roff (1973) would classify as a valid test. However, it would be foolish to totally discard the analysis on this basis, and to achieve any idea of the population size is of great use compared to none at all. From examination of the coefficient of variation, however, it is apparent that certain months should be discarded. These are April - June 1980, December 1980 - May 1981, and November 1981. For these months the value of the coefficient is high and even exceeds unity on three occasions! These are the winter months when the catch size was very low (see 4.2), and thus the requirement of a sampling intensity of 0.1 is not achieved. The only exception is June

1980, when although the sampling intensity was high, bias was introduced causing an overestimate of the population size since few animals were returned. This is reflected by the high standard error.

The values obtained for sampling intensity ($n_i/\hat{N}_i$) similarly indicate that the results for those months discussed above are likely to be inaccurate. A sampling intensity of greater than, or equal to 0.1 was only achieved during May, July and September of 1980, and July and August of 1981. It appears therefore that only those estimates of population size based on the summer months may be considered as being reliable. The results obtained in 1981 tend to have a lower coefficient of variation than 1980, and thus may be considered to be more representative of the actual situation. This is because in 1981 more marked animals were at risk (i.e., available for capture), and consequently the proportion of m_i is greater, lending more accuracy to the estimates. This point is illustrated in Fig. 3.7 which shows that the proportion of marked animals which are recaptured increases over the length of the two year study period.

During the summer months of 1980 Table 4.2 indicates that considerably more variation occurs in the population estimates than during 1981. In 1980 the range is from 386 in July to 2,159 in August, whilst in 1981 the range was 420 in August (2) to 1005 in September. The most accurate results for both years, however, based on consideration of sampling intensity and the coefficient of variation, tend to be nearer the lower figure.

Based on the criteria of Robson and Reiger (1964) (see 4.1 Introduction), the accuracy encountered in this study is insufficient

to permit detailed analysis of the population. With this in mind, only a brief consideration is afforded to the estimates of gain rate ($\hat{\beta}$) and survival ($\hat{\phi}$) which may themselves be subject to greater errors than the population estimate. The following points must also be considered; $\hat{\phi}$ should theoretically never exceed unity. If it does, then the only correct interpretation is that the value obtained is a combination of the true survival rate plus a positive error, and that the best estimate of should be considered to be 1.0, i.e. 100%. Similarly, $\hat{\beta}$ should not be negative, so any negative values are best considered to be zero, i.e. no gains to the population. The negative sign does not indicate a loss to the population (Begon, 1979).

The highest survival rates occur for the quarterly catch data e.g. July 1 to July 2 etc.. This is to be expected since they refer to an interval of only two days, whilst all other data relates to one month. Since $\hat{\phi}$ and $\hat{\beta}$ are subject to considerable error, further discussion of their values is confined to the summer months of 1981 when the most accurate estimates may be expected. The values for July 1981 day 2 are ignored due to the unusually high survival rate from day 1 to day 2 which has meant that the values of $\hat{\phi}$ and $\hat{\beta}$ do not compare directly with the other data based over a timescale of one month. It may be seen that survival of marked animals is consistently high from June to September, and decreases thereafter. Temperatures are falling from September onwards and so this may be explained by increased mortalities, or possibly decreased catchability of the larger animals (see 4.2) which tend to be marked.

Examination of the gain rate, $\hat{\beta}$, reveals no additions to

the population until August. More gains to the population occur in September, and again in October. This is to be expected since the July recruitment will not be included in the estimates achieved by the model until they reach 10 mm (C.L.) and are large enough to be marked. This size may be achieved in the latter months of the Summer and Autumn.

The other models of population size employed gave a variety of different results. The Jolly model provided an estimate of the actual population size over a period of several months, whilst the other models refer to a more discrete unit in time. It is probable therefore that the estimates achieved tend to reflect the potential catchable population at that time, rather than providing an estimate of the total population. Catchability is positively correlated to temperature (see 4.2) and so during the winter the potential population which may be caught may be less than the real population which exists, but is inactive and therefore excluded from the estimate.

Considering firstly the weighted mean and Petersen estimate, it may be seen from table 4.3 that in each case the coefficient of variability is a reasonable value, but that the accuracy of the Petersen estimate based on sampling intensity is only between 25% and 50% (Roff 1973). Certainly the values obtained are sufficient to provide a reasonable estimate of the population size. Seasonally it is apparent that higher catches are achieved during the summer when there are higher temperatures, and comparing the two study years, the population size tends to be greater in 1980 than 1981 (682 and 543 in July and September 1980, respectively, and 421 and 358 in July and August 1981 respectively,

based on the weighted mean).

Analysis of the results of the triple catch method reveal from the coefficient of variability that all estimates except August 1981 are highly questionable and should be discarded. However, they do serve to illustrate the same trends as observed previously, i.e. that the population size is smaller in 1981 than 1980.

Thus, in conclusion, it appears that from consideration of all the results, the absolute population size is best estimated during the summer when the largest proportion of the population are active and may be caught. The population size in 1980 is greater than in 1981, which is also reflected in the smaller catch sizes achieved during the latter year (see 4.2). These estimates refer only to animals of 10 mm (C.L.) and above, and therefore recruitment is not directly indicated in the values of population size. During the winter the potential catchable population is seen to decline. This may reflect some mortality, but is also due to the lower temperatures resulting in decreased activity of the crayfish. To summarize the estimates presented above and give an actual value to population size, two of the models are probably more reliable than the others. The weighted mean gave an average population size of 613 in 1980 for the two most accurate consecutive sampling periods, and the equivalent mean population size in 1981 was 390. These are likely to be underestimates (see Discussion), and the Jolly method is probably more accurate. For this method the estimates of the summer months during 1981 are highly plausible when a reasonably consistent population size was achieved, and both sampling intensity and

the number of marked recaptured animals were high. The same situation did not apply in 1980 when fewer marked animals were at risk, and so the estimate for this year is likely to be less accurate, and may be an overestimate. A comparison of the population estimates achieved during the summer months shows a mean population of 1071 during 1980, and 646 during 1981. Relating this to the area of the habitat (5 m wide, 60 m long, see 1.1), the following population densities result; 2 crayfish per square metre in 1980, and 1.3 m^{-2} in 1981 for the weighted mean estimates, and 3.6 m^{-2} and 2.2 m^{-2} in 1980 and 1981 respectively, for the Jolly estimates. It must be noted that this does not refer to the absolute density encountered per square metre, since clumping of the population tends to occur wherever hides are available, in this case, at the weirs.

4.1(iv) DISCUSSION

It was stated above that any interpretation of the estimates of the size of a population must be viewed within the limitations of the model used. That of Jolly and the triple catch model (essentially a special case of the Jolly model), require relatively few assumptions to be made. The Petersen estimate and weighted mean require more assumptions. However, all of the models are limited to the constraints imposed by the first five assumptions detailed above (see Introduction - Begon, 1979). The first stated that all marks must be permanent. If they are not, or fail to be recorded, then $\hat{N}$ will tend to be an overestimate and $\hat{\Phi}$ an underestimate. In this study the method of marking by cauterization has been shown to survive over several successive moults (see 2.2), and in addition all marks were reinforced on subsequent

recaptures. Consequently it is felt that this criterion is met, although when considering the use of Jolly's model, it is feasible that marks may have been lost over a two year period.

The second requirement of mark-recapture models concerned the effect of marking on catchability and survival. The former is unaffected since the sampling method does not require seeing the animals prior to capture, which may have resulted in a bias. Hence problems of underestimation of the population size resulting from increased catchability of the marked subpopulation do not arise. If survival is affected, and is decreased, then $\hat{N}$ will again be an underestimate, but $\hat{\phi}$, the survival rate, will be a good estimate since it is based solely on data from marked individuals. In the Leen population, however, predation was felt to be low (see 1.2v), and also the nocturnal nature of the crayfish would mean that the marks should not be a disadvantage in this respect. Mortalities arising from the marking technique itself are also extremely low, if indeed they occur at all (see 2.2). Thus criterion two is met.

Total mixing of marked and unmarked animals was the third criterion, in the absence of which underestimation of the population size is likely to occur. In considering the local distribution of the River Leen crayfish it was concluded that there was a tendancy towards the existence of a home range (see 3.3) and animals were often repeatedly found at the same site. "Clumping" of the population also occurs, with animals favouring areas with hides. Hence random mixing of the population may not occur. However, it must be noted that the sampling method specifically involved collections from 'clumped' areas, and within these there is no

reason to suggest that complete mixing does not occur since territoriality was not concluded to be of great significance. However, it must therefore be realised that criterion three may not be met, and particularly with the methods which involve only two days to allow mixing. Thus some underestimation of the population size may result.

It is necessary that the sampling period is short (criterion four) in relation to the total time in order that the estimates achieved may be related to an actual moment in time. This criterion is met for all of the models employed by this author. The fifth, however, may not be: that all animals are equally catchable. When this is not met, $\hat{N}$ tends to be underestimated whilst $\hat{\phi}$ is unaffected. Considering the population structure observed in the Leen (see 4.2) it is evident that certain subpopulations are less catchable than others and that this may vary seasonally.

Related to this assumption is the sixth, that all individuals have an equal chance of survival. If violated, $\hat{N}$ and $\hat{\phi}$ may not be affected, but it is questionable, however, as to the usefulness of an average survival rate for the whole population, when it is known that differential rates occur for the subpopulations. Concerning survival, the Jolly model further requires that survival be independent of age, which it is not. It appears then that it may be more useful to consider a series of subpopulations and to estimate separate values for $\hat{N}$. However, in this study insufficient recaptures were made to enable such treatment of the results, and it was necessary to consider the population as one complete unit. The low recapture rate achieved might have indicated that use of the Fisher-Ford model would be more beneficial,

but this would have necessitated the further assumption that survival was constant. Since this is not the case, it was felt better to employ the Jolly model. The Manly-Parr model requires neither of the latter assumptions about survival but more data are necessary than were available for the Leen population.

A further assumption applies to the Petersen estimate and weighted mean model: that the population is closed. Clearly it is not, leading to the possibility of overestimating the population size. The study area is not discrete and does not strictly, therefore, contain a 'population'. It was hence necessary to define artificial borders delimiting the 'population' to be studied. Begon (1979) argues that this is justifiable if the borders themselves are well defined. In this case a change in substrate was used to delimit the study area. At either end of the rocky, stony substrate of the study area were sections of the river containing sand/silt substrates with very few hides which may prove to be a natural barrier against migration out of the area (see 3.3), certainly in the short term, but less so over a two year period. Also evidence for a home range would imply that the same discrete population was being sampled meeting the requirements of mark-recapture models.

A comparison of the real situation with that assumed in the model thus reveals several shortcomings. The Jolly method may tend to underestimate $\hat{N}$, and the triple catch method even more so. Catchability over the short period of the triple catch was seen to decline, probably due to disturbance of the habitat resulting in successively smaller sample sizes. Possibly also it was the case that insufficient time was available for complete

mixing of the population when this model was employed and both of these factors would tend to produce an underestimate of $\hat{N}$. By contrast, Petersen and weighted mean estimates may tend to produce overestimates of the population size due to the possible occurrence of emigration and immigration, unless, if as was suggested for the triple catch method, mixing was incomplete, in which case an underestimate will result. It appears therefore that in each case any interpretation of the results must be guarded due to the failure to meet all of the requirements of the models. To discard the results completely, however, would deny any estimate of population size, and, providing that the limitations are understood, even these estimates may be safely employed in discussion of the population dynamics of the River Leen crayfish.

In short, due to the poor match between the required and real situations, analysis by the triple catch method may effectively be excluded. The weighted mean is similar to the Petersen estimate but is probably more accurate. Both underestimate the population size. The Jolly model provides the most accurate estimation of population size, but only in summer 1981 when both the recapture rates and the sampling intensity were high. The estimate of 646 achieved for this year is considered to be a reasonable representation of the true population size. For 1980 the estimates are less reliable but both the Jolly and weighted mean models show a higher population size in that year. Similarly estimates of survival ($\hat{\phi}$) and gain ($\hat{\beta}$) to the population were only considered to be worthy of discussion for the 1981 data. Survival was high throughout the summer but decreased towards the winter, probably due to temperature dependent mortalities. The gains to the population

revealed the new recruitment of the year, and were not observed until September when some of the juveniles had grown to 10 mm (C.L.) and were thus included in the population estimates.

The population density obtained for *A. pallipes* in Markfield Quarry was largely subjective. It is a reasonably high density, but this is consistent with the large number of hides available which are probably one of the most important factors in governing the potential maximum density of a crayfish population (see 3.3). The value of 6-8 m^{-2} given does not apply across the whole of the Quarry where the subtstrate is variable (see 1.2) but only to the population study area. Compared to the study area in the River Leen, the density encountered at Markfield Quarry is between two and three times greater (2.2 - 3.6 m-2 in Leen). Similarly, high population densities have been reported for *A. pallipes* in a Northumberland Aqueduct (3.8 - 10.4 m^{-2}, $\geq$13 mm C.L.), whilst the values reported for other river populations are more similar to those encountered in the Leen. Davies (1964) reported a density of 4 crayfish per square yard ($\approx m^{-2}$) in the River Brett, and Laurent (1972) reported 2 m^{-2} in the River Gournaz in France. Another study in France, in the River Mouliot, reported different population densities for a variety of microhabitats within one stretch of the river. They varied from 0.4 m^{-2} to 14 m^{-2} depending upon the availability of suitable hides. Tree roots were found to be preferred (Daguerre de Hureax and Roquello, 1981). The smallest population of *A. pallipes* recorded, is that of White Lake in Ireland, but it was based on a single population estimate, and then applied to the whole area of the lake, not taking into account density variations due to substrate differences. The density was 1,200

crayfish per hectare, equivalent to 0.12 m^{-2}. This compares with the strip census estimates made in Markfield Quarry which were thought to be underestimates. Comparison with these other observations supports the view that the estimates achieved for the two Midland populations are reasonable.

Variations in population density other than seasonal variation have been reported (Duffield, 1933, 1936; Pixell-Goodrich, 1956). Such long term fluctuations may result from a variety of factors such as changes in the food supply, or disease, and they may be density dependent. Feedback will result amongst the various processes involved such that regulation of the population size occurs (Solomon, 1976). Erradication of populations should only occur under abnormal conditions where feedback cannot occur, such as in cases of pollution, or, for example, with the introduction of the crayfish plague to which European crayfish are 'over-susceptible' (Unestam, 1972). Normally the feedback between the population size and the factors controlling it ensure that it remains a fairly constant size. Fluctuations only tend to occur when something adversely affects one of the dependent factors in the relationship.

For *A. pallipes* the fluctuations observed by the authors mentioned above, have been attributed to fungi, bacteria, protozoan disease and predation. Pixell-Goodrich (1956) suggests that an epidemic disease which destroyed crayfish near Oxfordshire (Calman, 1911) was probably *T. contejeanii* although this parasite has been seen to exist sympatrically with its host over a period of several years in Northumberland (Brown, 1979), and also in Midlands populations (Holdich *et. al.*, 1978). Whether it may reach "plague"

proportions or not, it is certainly possible that *T. contejeanii* may result in fluctuations of population density. In the River Leen a decrease in population size was observed in the study area from 1980 to 1981. The frequency of *T. contejeanii* was seen to increase over this period from 1.3% of the total population caught in 1980 to 6.2% in 1981 (see 4.2). Hence it may be possible to attribute this decline in population to the increase of the disease. The water quality over the two years was similar, and the substrate unchanged, both factors which have been shown to affect the distribution of crayfish (see 3.3), but which are not considered to have contributed to the decline in population. An alternative explanation could be that the cumulative disturbance to the habitat over the two year study period resulted in the displacement of some animals, which was manifest in the results of 1981, and reflected as a decrease in population size. However, it is felt that this is unlikely in view of the fact that marked animals were seen to remain within the area in which they were originally caught (see 3.3). Also, the period of disturbance was negligible compared to the time when the author was not at the population study site, and in addition it was ensured that the habitat was left in a similar condition as prior to sampling. Indeed, the weirs tended to be increased over the study period which should have enabled the support of a larger crayfish population. Thus it appears that the fluctuation observed is due to a natural cause, and this may be the parasite *T. contejeanii*. No evidence was found to suggest fluctuation in the numbers of crayfish at Markfield Quarry.

Finally, regarding the suggestion made in 3.1 that *A. pallipes*

might form a viable food resource, it is apparent that in the River Leen the population would very soon be exhausted, since it is relatively small. At Markfield Quarry the large population (of Ca 15,265) could potentially be utilized. However, to form a viable crop it is necessary to know not only the size of the population, but also the proportion of animals at a suitable age for cropping. Hence this point is discussed more fully elsewhere (see 4.2).

TABLE 4.1 A TRELLIS DIAGRAM FOR CALCULATION OF POPULATION SIZE BY THE JOLLY METHOD FOR THE LEEN POPULATION STUDY AREA

DATE OF SAMPLING OCCASION (i)	i	NO. CAPTURED Ni	NO. RELEASED ri	SAMPLING OCCASION; RECAPTURED MARKS (j) (NUMBERS IN PARENTHESES ARE RELEASE TIMES)																																
				(1) 2	(2) 3	(3) 4	(4) 5	(5) 6	(6) 7	(7) 8	(8) 9	(9) 10	(10) 11	(11) 12	(12) 13	(13) 14	(14) 15	(15) 16	(16) 17	(17) 18	(18) 19	(19) 20	(20) 21	(21) 22	(22) 23	(23) 24	(24) 25	(25) 26	(26) 27	(27) 28	(28) 29	(29) 30	(30) 31	(31) 32
DEC. 79	1	8	8																																	
JAN. 80	2	9	9	0																																
FEB	3	10	10	0	0																															
MAR	4	27	27	1	0	0																														
APR	5	29	29	0	0	0	0																													
MAY	6	52	51	0	0	0	1	3																												
JUN	7	93	37	0	0	0	0	1	1																											
JUL 1	8	100	99	0	0	0	1	0	2	4																										
JUL 2	9	65	64	0	0	0	0	0	0	2	12																									
AUG	10	95	24	0	0	0	0	0	0	0	1	3																								
SEPT 1	11	56	56	0	0	0	0	0	0	0	3	0	7																							
SEPT 2	12	53	51	0	0	0	0	0	0	0	0	0	4	5																						
OCT	13	63	60	0	0	0	0	0	0	0	1	0	0	4	3																					
NOV	14	54	54	0	0	0	0	0	0	0	0	3	0	2	0	1																				
DEC. 80	15	16	16	0	0	0	0	0	0	0	0	0	0	0	0	0	1																			
JAN 1 81	16	16	16	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0																		
JAN 2	17	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3																	
FEB	18	13	13	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0																
MAR	19	12	12	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	3															
APR 1	20	14	14	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0														
APR 2	21	12	12	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3													
MAY	22	23	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0												
JUN	23	45	45	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2											
JUL 1	24	65	61	0	0	0	0	0	0	0	2	0	1	1	0	0	2	0	0	0	0	1	0	0	1	5										
JUL 2	25	42	41	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	10									
AUG 1	26	65	64	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	4	2								
AUG 2	27	81	78	0	0	0	0	0	0	0	0	0	1	0	1	2	0	1	0	0	1	0	2	0	2	2	2	1	14							
SEPT	28	55	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	3	0	2	4						
OCT 1	29	36	35	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2					
OCT 2	30	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	4				
NOV	31	22	22	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0			
DEC	32	14	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1			

TABLE 4.2 POPULATION ESTIMATES FOR THE RIVER LEEN
USING JOLLY'S (1965) STOCHASTIC MODEL

MONTH	i	n_i	r_i	m_i	Y_i	Z_i	$\hat{M}_i$	$\hat{N}_i$	$SE\hat{N}_i$	$\frac{SE\hat{N}_i}{\hat{N}_i}$	$\hat{\phi}_i$	$\hat{\beta}_i$	$\frac{n_i}{\hat{N}_i}$
DEC	1	8	8	-	1	-	-	-	-	-	-	-	-
JAN	2	9	9	0	0	1	-	-	-	-	-	-	-
FEB	3	10	10	0	0	1	-	-	-	-	-	-	-
MAR	4	27	27	1	2	0	-	-	-	-	-	-	-
APR	5	29	29	0	5	2	11.60	348	-	-	0.8522	70	0.08
MAY	6	52	51	4	5	3	34.60	367	275	0.75	0.4779	1,047	0.14
JUN	7	93	37	2	6	6	39.00	1,222	1,020	0.83	0.4131	-119	0.08
JUL 1	8	100	99	7	21	5	30.57	386	177	0.46	1.009	155	0.26
JUL 2	9	65	64	14	7	12	123.71	544	247	0.45	0.6474	1,807	0.12
AUG	10	95	94	4	13	15	112.46	2,159	1,264	0.59	0.4050	-449	0.04
SEP 1	11	56	56	10	14	18	82.00	425	161	0.38	2.3613	628	0.13
SEP 2	12	53	51	9	4	23	302.25	1,632	972	0.60	0.4465	364	0.03
OCT	13	63	60	8	7	17	153.17	1,093	559	0.51	0.7792	407	0.06
NOV	14	54	54	6	7	20	160.29	1,259	685	0.54	0.3376	173	0.04
DEC	15	16	16	1	6	26	70.33	598	595	0.99	0.9804	125	0.03
JAN 1	16	16	16	1	6	31	83.66	711	724	1.02	5.1996	-1645	0.02
JAN 2	17	15	15	3	1	34	513.00	2,052	2,260	1.10	0.1672	66	0.01
FEB	18	13	13	2	5	33	87.80	409	302	0.74	3.9474	-890	0.03
MAR	19	12	12	6	1	32	390.00	724	723	0.99	0.2242	282	0.02
APR 1	20	14	14	2	5	31	88.8	444	327	0.74	1.0361	318	0.03
APR 2	21	12	12	5	0	31	-	-	-	-	-	-	-
MAY	22	23	23	2	7	29	97.29	778	582	0.75	0.7360	94	0.03
JUN	23	45	45	5	17	31	87.06	667	310	0.46	0.9424	-64	0.07
JUL 1	24	65	61	13	20	35	119.75	565	174	0.31	1.8730	-383	0.12
JUL 2	25	42	41	19	5	36	314.20	675	304	0.45	0.3522	320	0.06
AUG 1	26	65	64	13	17	28	118.41	558	184	0.33	0.9079	087	0.12
AUG 2	27	81	78	29	10	16	153.80	420	140	0.33	1.2393	484	0.19
SEP	28	55	55	13	3	13	251.33	1,005	638	0.63	0.3452	277	0.05
OCT 1	29	36	35	5	4	11	101.25	624	410	0.66	0.3809	-74	0.06
OCT 2	30	15	15	7	0	8	-	-	-	-	-	-	-
NOV	31	22	22	6	1	2	50	164	179	1.09	-	-	0.14
DEC	32	14	-	3	-	-	-	-	-	-	-	-	-

TABLE 4.3 A SUMMARY OF THE QUARTERLY POPULATION ESTIMATES OBTAINED USING THREE DIFFERENT MODELS (R. LEEN)

RECAPTURE DATA USED FOR ESTIMATES									POPULATION ESTIMATES: METHOD =;												
DATE OF SAMPLING OCCASION	NUMBER CAUGHT (1) n_i	NUMBER RELEASED r_i	NUMBER RECAPTURED (2) n_2	NUMBER MARKED m_{21}	NUMBER RELEASED r_2	NUMBER RECAPTURED (3) n_3	NUMBER MARKED (1) m_{31}	NUMBER MARKED (2) M_{32}	PETERSEN ESTIMATE		WEIGHTED MEAN		TRIPLE CATCH METHOD					JOLLY'S STOCHASTIC MODEL					
									$\hat{N}_1$	$SE\hat{N}$	$\hat{N}_{1-3}$	$SE\hat{N}$	$\hat{M}_2$	$\hat{N}_2$	$SE_{\hat{N}2}$	$\phi_{N1\to2}$	$s_{2\to3}$	$\hat{M}_i$	$\hat{N}_i$	$SE\hat{N}_i$	ϕ_i	s_i	i
JULY 1980	100	100	65	5	65	47	2	4	471	108	682	161	39.4	186	117	0.39	0.69	30.6	386	177	1.00	155	1
																		123.7	544	247	0.65	1,807	2
SEPT. 1980	55	55	53	5	55	35	3	3	495	176	543	172	45.5	410	348	0.83	-0.17	82.0	425	161	2.36	628	1
																		302.3	1,632	972	0.45	364	2
JAN. 1981	16	16	15	3	15	3	1	1	64	25	54	27	37	148	-	2.31	-1.50	83.6	711	724	5.20	-1645	1
																		513.0	2,052	2,260	0.17	66	2
APRIL 1981	14	14	12	3	12	11	-	1	46	17	84	48	-	-	-	-	-	88.8	444	327	1.04	318	1
																		-	-	-	-	-	2
JULY 1981	65	62	42	10	42	44	2	3	242	60	421	113	31.5	123	84	0.51	0.72	119.8	565	174	1.87	-383	1
																		314.2	675	304	0.35	320	2
AUGUST 1981	65	65	81	13	81	33	2	11	381	90	358	72	26.7	156	63	0.41	0.45	118.4	558	184	0.91	-87	1
																		153.8	420	140	1.24	484	2
OCTOBER 1981	35	35	15	4	15	10	-	-	112	38	197	113	-	-	-	-	-	101.3	624	410	0.38	-74	1
																							2

4.2 POPULATION STRUCTURE

4.2(i) INTRODUCTION

The term 'population structure' in the context of this thesis is taken to include both the make-up of the population in terms of numbers of animals of a particular type (i.e. immature, mature, male, female, etc.) and also seasonal variations in these observations. The structure of the population may affect fecundity of the total population (i.e. numbers of males to females, see 3.2) or juvenile survival through competition with mature animals. It is also important when considering the feasibility of exploiting a particular stock since males are preferred due to their larger chelae, and it will also be important to know what proportion of a particular stock has lost its chelae or is diseased, for example. The following section is a presentation of such data collected for the River Leen and Markfield Quarry populations.

4.2(ii) METHODS

Every animal collected from each population study site was subjected to a series of measurements and observations as described previously (see 2.1-2.3). The data was stored in the Nottingham University ICL 2900 computer and was analysed using the SPSS statistical packages. Having coded each observation it was possible to withdraw from the computer files all data with a particular code, for example, all diseased animals, and thus it was possible to examine the population structure. The parameters examined were:

(i) Seasonal variation in numbers caught and the age structure of the population each month.

(ii) Sex ratios, and their seasonal variation.

(iii) The proportion of immature:mature animals seasonally.

(iv) The frequency of diseased animals.

(v) The frequency of damaged animals (i.e. chelae missing, damaged exoskeleton).

In relation to point (ii), the seasonal variation of sex ratios, a laboratory feeding trial was also conducted around the time of hatching in order to elucidate whether a reduction in foraging activity, and therefore in catchability also, might be occurring for ovigerous females. These results are also presented, the experimental procedure being as follows.

75 ovigerous females of the Nanpantan Reservoir stock were maintained individually as previously described (see 3.2). A control of 20 non-ovigerous females of a similar size range were subjected to the same regime and all animals were fed twice weekly. The experiment was started in March, ten weeks prior to hatching, and ceased six weeks after hatching was completed. The first feed consisted of trout pellets whilst the second was raw ox liver. The liver was cut into cubes and weighed accurately to 0.01g (each cube was approximately 1g) so that the precise weight of liver given to each individually numbered crayfish was known. After 24 hours it was removed, dried with tissue paper and reweighed. Since a weight increase was observed due to the uptake of water, 20 control pieces of liver, which were not presented to any animals, were also treated similarly and the mean percentage increase in weight was recorded (usually 15-20%) and a correction factor was applied to the original weights of liver used experimentally. By subtraction, the amount of liver consumed per animal was determined. Results indicating that none had been eaten were

easily confirmed since the appearance of eaten and uneaten liver was quite different. After the eggs had hatched the females were weighed enabling the results to be expressed as a mean percentage of the total body weight consumed per week.

4.2(iii) RESULTS

Fig. 4.1 shows the total numbers of crayfish caught each month of the study period from the River Leen population study area. The maximum number of animals caught occurred in July 1980 (265 animals) and the minimum was in January of that year (10 animals). A similar trend occurs in 1981. Part of the reason for the dramatic variation observed between January (winter) and July (summer) is the new recruitment which occurs in July when large numbers of newly hatched juveniles are caught (represented by shaded area in Fig. 4.1). However, when recruitment is excluded the variation in catch remains. Fig. 4.2 shows log. catch plotted against temperature and excludes the new recruitment (i.e. it is based only on animals of carapace length 10 mm and greater). A positive correlation is found to exist with increasing temperature ($r = 0.7898$) indicating that this factor in addition to new recruitment also explains the seasonal variations in catch that are observed.

Fig. 4.3 shows the corresponding catch data for Markfield Quarry collected over the study period, where the method of capture was by trapping. Again it is seen that the maximum catches occur during the summer months (July 1981, 46 animals). The minimum in fact occurred during March when no animals were caught, but the trend is for lower catches during the winter months. Plotting log. catch against temperature reveals a highly significant positive

correlation ($r = 0.9182$), the catch size increasing with increasing temperature (Fig. 4.4). The catch of one animal during November 1981 was excluded from Fig. 4.4 since the traps had been tampered with allowing the crayfish to escape.

Unlike the Leen population, it was not possible to explain any of the observed variations in catch by recruitment, since no 0+ juveniles were ever caught at Markfield Quarry. For the River Leen population recruitment has been illustrated in Fig. 4.5 which shows the proportion of the total catch for each month which may be explained by the 0+ year class. It may be seen that the proportion of juveniles in the catch during July (after hatching has occurred) is very high, being 62.5% and 70.5% for 1980 and 1981 respectively. This falls rapidly off to around 15% over the winter months of 1979/80, and 1980/81. However, for the winter period 1981/82 the proportion of juveniles in the catch remained relatively high (40-50%). A possible explanation for this is presented in the discussion.

In order to examine the age structure of the River Leen population each month for the older year classes, in addition to the 0+ year class, polymodal size frequency analysis was conducted (Cassie, 1954). Details of the method employed and the results achieved are presented in 5.1 but a summary of the analysis is now presented since the results are of relevance at this point. Tables 4.4 and 4.5 show these results for males and females respectively. The limitations to this method of analysis which are posed during the summer months are discussed in 5.1, but despite these the results given show some interesting trends which are similar for males and females. One would expect to

find a decreasing proportion of the population represented by each subsequent age class. However, this is not the case, and the 0+ and 1+ year classes are under-represented. This may be explained by a difference in the catchability of this part of the population, which is more likely to be the result of behavioural differences than the fishing method employed, which is unselective. The age classes in question represent the smallest crayfish, and these may seek a different habitat from the larger ones found under the rocks and stones. Trap entry has been shown to be affected by dominance order (Brown, 1979), and so the favoured natural hides under large stones may be similarly affected. Since the method of sampling most extensively covers the weirs and rocky areas, a bias may exist towards the larger animals.

Recruitment is apparent in Tables 4.4 and 4.5, and is seen in July when the 0+ age class suddenly increases to represent over 50% of the total population caught. At the other extreme it may be seen that the proportion of the catch represented by the older (and larger) animals is very low, usually less than 5%. It must also be remembered that the figure of 5% represents all the age classes between 4+ (or sometimes 5+), and the oldest animals at about 10+. Thus the proportion of animals actually in their tenth year must be very small. Similar analyses for the Markfield Quarry data were not conducted, for reasons outlined in 5.1.

In addition to recruitment and temperature variations, some of the seasonal differences observed in the total catch may be explained by seasonal variations in the catchability of certain sub-populations. For example, ovigerous females may be less

vulnerable to capture. In order to test this, both the monthly sex ratios, and the proportion of mature (>25 mm C.L.) to immature (<25 mm C.L.) males and females caught each month were examined.

The sex ratios for all the animals of carapace length greater than 10 mm which were caught during the study period was considered first for each population. During 1980 293 males and 318 females were caught in the River Leen. In 1981 there were 206 males and 214 females caught. Expressed as male ÷ female, this gives ratios of 0.92 and 0.96 for the two consecutive years. A ratio of unity occurs when equal numbers of each sex are caught, of less than unity when more females are caught, and of greater than unity when more males are caught. Thus the resultant ratios indicate approximately equal numbers of each sex (as seen from the raw data), but with slightly more females caught. When considering the sex ratios exhibited by mature animals and immature animals it is similarly found that the sexes are represented almost equally although in 1980 rather more mature females than males were caught. The results were: 1980, mature animals, 0.89, immature animals, 0.95, and 1981, mature animals 0.98, immature animals 0.95.

At Markfield Quarry the sex ratio observed for the total number of animals caught by trapping was 1.99 (139 males, 70 females). This excess of males however does not represent the true sex ratio to be found in the population, but indicates the bias of the fishing method (trapping) towards males. The sex ratio obtained when only hand caught samples are considered (snorkelling) was 1.02 indicating approximately equal numbers of males and females in the population. A note was also made of the sex ratio exhibited by the Nanpantan stock caught during

November 1979 which was represented by 762 animals. Its value was 1.04 for the whole population, 1.03 for mature animals, and 1.08 for immature animals.

To examine any seasonal variation of sex ratios that might occur in the River Leen population, mature and immature animals were treated separately (Figs. 4.6, 4.7). For the Markfield Quarry population so few animals of carapace length less than 25 mm were caught that no distinction was made between mature and immature animals. The seasonal variation in sex ratio observed for this population is represented in Fig. 4.8. From Fig. 4.6 for mature animals from the River Leen it may be seen that there is considerable variation of the sex ratio throughout the study period. However the "loose" trends which become apparent over the two year period are that during the breeding season, which is indicated by the horizontal solid line, between arrows at the bottom of the graph, the ratio is in excess of unity indicating a higher proportion of males in the catch. Outside this season the sex ratio is seen to be less than unity, but from October to November of each year it suddenly increases, coinciding with the timing of egg laying. However, consideration of Fig. 4.7 for immature Leen animals reveals a similar trend with a low sex ratio outside the breeding season, suddenly increasing in November of 1980, and December of 1981. This casts doubt as to whether the trend observed for mature animals is actually explained by differences in the catchability of ovigerous females.

For Markfield Quarry it may be seen that the samples collected by trapping demonstrate a marked variation of sex ratios throughout the year (Fig. 4.8), whilst the hand caught samples (when

snorkelling) are all approximately equal to unity. The tendancy seen to occur for the trapped samples is for the sex ratio to be in excess of unity (an excess of males to females), and indeed it only falls below on three occasions. The first was Jan. 1981 and relates to only one animal, a female. The data for May 1981 however, is of more interest since 8 animals were caught, all of which were female and 3 of which were carrying eggs. This represents a catch that one would not expect due to the bias of trapping towards males. The fact that ovigerous females were caught also tends to support the view that the presence of eggs does not affect the behaviour of these females towards traps, as observed by Brown (1979). In Northumberland Brown (1979) found that the only time that the sex ratio of his trapped samples of *A. pallipes* fell below unity was in the month prior to egg laying. Similarly this was the case at Markfield Quarry, and the third occasion that the sex ratio was less than unity was during October 1981 prior to spawning in November when the ratio had increased again.

The question of whether the catchability of ovigerous females may affect the observed sex ratio throughout the year was also examined in the feeding trials, the results of which follow this section. A reduction in feeding activity is seen to occur around the time of hatching, and so this may explain why more males are caught at this time, as the foraging activity of the females ceases. The experimental results, however, tend to imply that this would only be the case at the precise time of hatching so monthly collection of data might be expected to miss this exact moment. Indeed, examination of Fig. 4.6 for mature Leen animals reveals

that in June 1980 the sex ratio is less than unity whilst in 1981 it is greater, implying that if decreased catchability of females does occur at hatching then that point in time was almost certainly missed during 1980. The greater number of females present in the catches during the summer months may be explained by increased foraging activity in order to meet the energetic demands of egg production in the autumn.

The structure of the River Leen population in terms of the proportion of mature animals to immature animals is expressed in Figs. 4.9 and 4.10 for males and females respectively, throughout the study period. For both sexes it will be seen that more mature animals are caught during the summer months. In the case of the females a possible explanation may again be the differences in catchability of egg bearing females. However, that the same differences occur for the males casts doubt upon this hypothesis, and the variations in the catchability of mature animals which are observed are perhaps explained by behavioural differences of a different nature possibly related to temperature (see Discussion). Pooling all results it is seen from the mean ratio of immature:mature animals that more immature animals were caught. The ratio in 1980 was 0.53:0.47, and in 1981 was 0.54:0.46 immature: mature animals, respectively. This is to be expected due to recruitment of juveniles into the stock at relatively high levels each year.

The frequency of diseased animals in each stock population is represented in Table 4.6. *Thelohania contejeani* Henneguy is a crayfish parasite known to affect populations of *A. pallipes* (Cossins, 1972). It is usually apparent when the abdominal muscle is viewed

through the transparent sternum. Normally the striated muscle blocks are themselves transparent, but when infested with *T. contejeani* they become white in appearance. The disease results in deterioration of muscle function leading to death, probably due to starvation since the crayfish is virtually rendered immobile (Holdich *et. al.*, 1978).

The 'closed' population at Markfield Quarry was found to be entirely free of this disease but it was observed in animals from the River Leen, and also those caught at Nanpantan Reservoir. Fig. 4.11 represents the size frequency diagram for all the diseased animals caught in the River Leen over the two year study period. It shows that all sizes of crayfish are susceptible to the disease, the smallest being two 13 mm (C.L.) females whilst the largest was a male of 45 mm (C.L.). In total 8 diseased animals were caught in 1980 (1.3% of the population), 2 of which were females. In 1981 the incidence of the disease had increased to 6.2% of the population when 26 affected animals were caught, half of which were males, and half females. From Table 4.6 it may be seen that a greater number of large animals are diseased than are small ones, which is probably a reflection upon the fact that they will have had more exposure to the disease than the immature animals. Similar proportions of males and females are infected with the disease. In total only 6 animals from Nanpantan Reservoir were found to be affected which represents 0.79% of the total population caught.

Fig. 4.12 shows the size frequency of damaged animals collected from the River Leen during the two year study period. It reveals that both sexes and all size classes of animals are liable to

some form of damaged exoskeleton. Table 4.7 analyses the frequency of damaged animals for each population in terms of mature and immature males and females. In each case all the animals collected throughout respective study periods are pooled for analysis, and the type of damage, and its frequency are recorded.

A higher incidence of damaged animals occurs in the River Leen population than Markfield Quarry, and more damaged animals were caught in 1981 than 1980 for the former site. Similar proportions of each sex are damaged, but for the 1981 Leen population more mature animals than immature animals are seen to be damaged. The proportion of mature and immature animals damaged in 1980 are similar. In every sub-population examined the most common type of damage observed is the loss or regeneration of one chela, which represents about 70-85% of all observations. Loss of both chelae is the next most common observation with damage to the rostrum or telson, for example, forming only a very minor proportion of the total observations.

FEEDING TRIALS WITH OVIGEROUS FEMALES

The experiments on the feeding behaviour of ovigerous and non-ovigerous females were conducted in the manner described above (see 4.2 Methods) and the results are expressed as the mean percentage of the total body weight which was consumed (of liver) each week. Fig. 4.13 shows these results against time for both ovigerous and non-ovigerous females, whilst Fig. 4.14 shows the proportion of animals which had eaten nothing in any one week. From Fig. 4.13 it may be seen that initially the non-ovigerous females appear to consume more liver per week than those bearing eggs. This slight difference, however, is not significant and

precisely the same trends in consumption of liver are followed by both sub-populations examined. Around the time of hatching, however, differences do become apparent and one week prior to this occasion the consumption of liver by non-ovigerous females rises whilst that of the ovigerous females is seen to decrease. At hatching the amount eaten by the egg bearing females is the minimum amount recorded throughout the entire experimental period (1.75 ± 2.97% of body weight), whilst that of the non-ovigerous females remains elevated. One week later both sets of females show an increase in the amount of liver consumed although that of the ovigerous females appears to be greater. Thereafter, the trends in feeding exhibited by the two sub-populations are, as prior to hatching, identical.

Thus from Fig. 4.13 it may be seen that for the actual week when hatching occurs, and for one week either side of this time, ovigerous females appear to consume less food. Closer examination reveals that this difference is highly significant at the time of hatching (ovig. = 1.75 ± 2.97%, non-ovig. = 6.25 ± 4.12%; $t = 3.33$, $P < 0.01 > 0.005$), whilst the periods prior to, and post hatching reveal less significant differences (prior to: ovig. = 3.27 ±3.95%, non-ovig. = 6.81 ±4.60%; $t = 2.32$, $P > 0.025$. Post: ovig. = 6.99 ±6.39%, non-ovig. = 14.02 ±8.33%; $t = 2.56$, $P > 0.025$). Fig. 4.14 also reveals that it is the actual period around hatching when differences between the two sub-populations may be observed, whilst at other times prior to, and post hatching, the trends are similar.

Figs. 4.15a. and b. show the log. mean percentage of liver consumed against temperature. Large variations in the amount

consumed per week are apparent from Fig. 4.13, and one might expect some of this variation to be explained by temperature differences, with more food being eaten the higher the temperature. However, this does not appear to be the case and no correlation was observed between the temperature and the amount of food consumed, for either sub-population; $r = 0.0987$, $P > 0.5$ for ovigerous females (Fig. 4.15a), and $r = 0.1025$, $P > 0.5$ for non-ovigerous females (Fig. 4.15b). The three weeks around hatching were excluded from the regression of Fig. 4.15b, but when plotted (0, -1, +1 on Fig. 4.15b) the value for the week of hatching in particular shows that a considerably smaller amount of liver was consumed than might be expected.

4.2(iv) DISCUSSION

This section concerned with the population structure of each study population has considered details such as the sex ratio of the population, the size class distribution, and the frequency of diseased and damaged animals encountered. It is apparent that although an overall value may be ascribed to these factors encompassing all the data collected throughout the study period, the most useful information may be achieved by examining them within sub-populations of mature and immature males and females. Furthermore, variations in the population structure of captured animals may occur seasonally which is related to the different 'catchabilities' of certain sub-populations throughout the year.

Catchability then, is of prime importance when considering the population structure. The seasonal variation in the total numbers caught, excluding recruitment, was shown for both of

the Midlands populations studied, to be correlated to water temperature. This is related to catchability in that with decreasing temperatures crayfish become less active, and foraging behaviour is reduced (Roberts, 1944; Morrissy, 1975). Hence they are less likely to be caught. Morriarty (1971) obtained an unusually high catch of *A. pallipes* during December but explained this by stating that the crayfish were searching for suitable hides in which to hibernate over the following cold months. Whether hibernation as such occurs or not it was certainly noticed by this author that all animals caught during the coldest part of the year were moribund and extremely innactive. A decrease in the numbers of *A. pallipes* caught during the winter has also been observed for other British populations (Thomas and Ingle, 1971; Brown, 1979).

Seasonal variations in the sex ratio may also be explained by differences in catchability. The bias exhibited towards large males caught by trapping (Brown, 1979) explains the abnormal sex ratios reported for trapped samples from Markfield Quarry. Hand collected samples from both sites, however, had sex ratios (taken over the whole year) which did not differ significantly from unity. Seasonally variation did occur, and at the River Leen it was seen that for mature animals more males were caught during the breeding season, whilst more females tended to be caught for the rest of the year, the summer. The trapped samples of Markfield Quarry were subject to bias, but showed that in September and October the sex ratio was approximately unity, whilst in November after egg laying it was in favour of males. Morriarty (1971) found that females outnumbered males in August and September, prior to the breeding season, but the reverse

was true for the rest of the year with his trapped samples. Similarly, Brown (1979) also reports more females in trapped samples prior to egg laying. This trend was also observed in the hand collected samples of the River Leen where the ratio was observed to change dramatically from October to November in favour of males.

Variations in the sex ratio may then, it seems, be explained by reduced catchability of egg bearing females. Thomas and Ingle (1971) report that during June 1964 ovigerous females were not caught in their study area, but had migrated to a deeper part of the river. In the Leen such a migration is unlikely since the river does not become deep until a considerable distance beyond the confluence of the East and West branches where the water quality is unsuitable (see 3.3). A more likely explanation is that they had obtained more secure hides, perhaps under tree roots and the banks of the river, and where, therefore, they were less likely to be caught. They then remain relatively innactive during the brooding period. Such behaviour has also been reported for *Pacifastacus trowbridgii*, and it is suggested that this behaviour is of survival value for the developing eggs and juveniles (Mason, 1970).

The innactivity of the ovigerous females is reflected by reduced catchability. One of the prime reasons for locomotion by crayfish is food searching (Roberts, 1944) and so it is likely that ovigerous females may eat less during brooding. In this study this proved to be the case only for a period of three weeks around the time of hatching. This may have survival value, since if the desire for food is suppressed, then cannibalism of the

juveniles is less likely to occur. Tactile cues from the juveniles are suggested to cause this suppression (Little, 1976). These cues, through a feedback mechanism, also result in the production of a chemical brooding attractant by which the juveniles recognize the parent crayfish (Little, 1975, 1976). Non-ovigerous females, and males, do not produce this attractant, and will also tend to consume juvenile crayfish (Mason, 1970; Little, 1975, 1976). Total inhibition of feeding by ovigerous females was not reported, and in some species it is suggested that in the absence of the tactile feedback, once the juveniles have become free living, the behaviour of the brooding females may change to include juvenile cannibalism (Little, 1976).

The results reported here show that no reduction of feeding was observed for non-ovigerous females. That the reduction found to exist for brooding females only lasted for one week after hatching may be explained by the fact that all juveniles were stripped off the females for laboratory investigations. Hence, in the absence of tactile feedback, feeding may have been resumed earlier than would otherwise have been expected. These observations are of importance in studies of growth also. The evidence for feedback mechanisms may explain why moulting is suppressed in ovigerous females. Also the lower growth rates observed for females (see 5.1) have sometimes been attributed to their reduced feeding activity whilst brooding (Abrahamsson, 1972a). It may also be that the slightly higher catches of females compared to males during the summer months are due to their increased foraging activity in order to make up for any such reductions of feeding activity, and also to cope with the energetic demands of producing

the next brood.

Examination of the seasonal variation of sex ratios of immature animals casts doubt upon the hypothesis that the variations observed for mature animals result from differential catchabilities of males and non-ovigerous females, and the ovigerous females. It was found that a similar trend was exhibited as for the mature animals, but the trend was not so strongly shown. Neither was the change in the ratio, from more females to more males, which occurred at the end of the summer, consistent. It seems probable therefore that the differing catchability of ovigerous females will explain the pattern observed for mature animals. This is particularly so at the beginning and end of the brooding season when differences between the sexes were most pronounced.

Seasonal variation in the size structure, observed also for other populations of *A. pallipes* (Morriarty, 1971; Thomas and Ingle, 1971; Brown, 1979), was due chiefly to recruitment and mortality. The proportion of the Leen population represented by the 0+ year class rose dramatically each year from June to July (to around 65%) due to the new recruitment. In successive months the proportion of this class was seen to decline. For both the 0+ and 1+ year classes the proportion of the population that they represented was less than might be expected. Catchability is again the answer. The smallest animals tended to be caught amongst the allocthonous organic matter such as twigs and leaves that collected up against the weirs. Due to the large volume of this material it was not all systematically sorted, and consequently a bias may have resulted towards the larger animals which tended to be caught under the large rocks of the weirs.

Part of the decline in numbers representing the 0+ year class in successive months may be explained by mortalities. The largest difference, however, was observed from July to August and is again explained by differences in catchability resulting from behavioural differences over this period. When just hatched (July) the juveniles tended to group together and several would be caught together with an adult female. Hence a higher catch rate was achieved than during the following months when the juveniles will have left their parent and dispursed. The mortality rates experienced by the 0+ year class are not well documented due partly to the difficulties of catching this size of animal. For *Astacus astacus* Kaestner (1970) estimates that 20 juveniles per brood survive to the post-larval stage, whilst Kossakowski (1971) assumed that a mean number of 16 per brood would survive until the first autumn. Momot and Gowing (1977a) report 85-90% mortalities for the 0+ year class of *Orconectes virilis*. Lake and Newcombe (1975) also report high juvenile mortalities for *Parastacoides tasmanicus*, but the population structure differs from that in the Leen in that it was dominated by large slow growing mature animals, with few juveniles.

In the River Leen study area during 1980, the potential recruitment was estimated as 7,071 juveniles. Only 166 were caught in July of that year which represents only 2.35% of the potential. This was 66.2% of the total population caught that month. By the overwintering period this proportion had fallen considerably to around 15% indicating that considerable juvenile mortalities must occur. For the overwintering period of the previous year (1979/80) the proportion of 0+ juveniles was also around 15%,

but prior to that of 1981/82 it was not seen to decrease so rapidly and during December 1981 the 0+ year class still represented as much as 48% of the population. It appears then that juvenile mortality was not so great during 1981 as in the previous two years. There are three possible explanations for this. The first is that the temperatures were not so extreme that year. Abrahamsson (1972a) has shown that the survival of juveniles is correlated to temperature extremes. However, conditions were similar over the three winter periods studied (see 1.1) and so this is an unlikely explanation. the second explanation is that the author had become more experienced at collecting crayfish and recognized the juveniles more easily. This, however, it not considered to be the case since the period in question represents the third winter of sampling, and sufficient experience at sampling is felt to have been achieved by the second winter. Thus, the final explanation is considered to be correct, that survival is density dependent. Momot and Gowing (1977b) report that young of the year mortalities are directly related to the population density, as is fecundity (see also, Dye and Jones, 1974). In 1980 the population in the River Leen was greater than during 1981. High juvenile mortalities in the former year will therefore explain the low proportion of juveniles surviving to the overwintering stage. In 1981, despite the lower population density, the potential recruitment was estimated to be similar to 1980 (see 3.2(ii)), and due to reduced competition the juvenile survival is also seen to be greater. Hence it may be seen that the capacity exists within the population to regulate and maintain its size.

For the animals represented in the 2+ age class and greater,

a decrease in the proportion of the population represented by each year class occurs. This is explained by natural mortalities which, as for the juvenile population, are density dependent (Momot and Gowing, 1977b). In addition to the environmental factors such as temperature, food availability, shelter, and population density, which affect crayfish mortality, other losses may be explained by moulting failures and cannibalism (Momot, 1967). Indeed, Flint (1975) suggests that mortality increases exponentially with age. In the Leen population the small numbers of large animals is quite marked, and at Markfield Quarry although a bias exists towards the larger animals, the proportion of the very large older crayfish is extremely small. These observations are of relevance in considering the cropping potential of a population, since although the actual population size may be large, the biomass represented by animals of a marketable size will only be small.

Seasonal variation was also seen to exist for the relative numbers of mature animals caught. For both males and females the tendancy was for more mature animals to be caught during the summer months than the winter. The trend was more apparent for males than females, and the population of mature males tended towards a maximum in October, falling off rapidly after this time. This is perhaps related to seasonal temperature differences in that the large dominant males defend the prime overwintering hides (Bovbjerg and Stephens, 1974), and are thus more difficult to catch. As the temperature increases they become more active, and their relative proportions increase in the catch. The trend exhibited by the females may be explained due to the seasonal variation in catchability which occurs for ovigerous animals,

and the tendancy for the proportion of mature animals to approach a maximum in October may be explained by sexual behaviour. During October fertilization occurs. Chemical communication between the sexes (see Gaudioso Lacasa and de Paz Cabello, 1979) at mating time may also tend to make mature animals more active, and therefore more susceptible to capture.

The frequency of animals with damaged or regenerating limbs, and with the disease *T. conjeanii* was also reported. Abrahamsson (1966) reported that a high population density resulted in a high frequency of animals with missing chelae. This might be expected due to the aggressive nature of crayfish (Bovbjerg, 1956; Bovbjerg and Stephens, 1974; Rubenstein and Hazlett, 1974). The chelae are employed in sexual displays, although during the act of copulation *A. pallipes* is far less aggressive than reported for other species (Ingle and Thomas, 1974). Hence most chela loss is likely to be confined to aggressive and defensive actions which would increase with populations of greater density. Contrary to this hypothesis, however, it was found that the frequency of damaged animals was the least for the Markfield Quarry population which has the greatest population density, and it was greater during 1981 than 1980 in the River Leen. During 1980 the Leen population was greater than in 1981. The explanation lies in the fact that Markfield Quarry offers a greater density of potential hides and so aggressive interactions may consequently be less than in the Leen. It is, however, more difficult to explain the frequencies observed over the two years in the Leen. It may be that the higher frequency in 1981 is a reflection of the limb loss initiated the previous year.

Thelohania contejeanii is a microsporidian endoparasite of *A. pallipes* (Cossins, 1972; Vey and Vego, 1972; Cossins and Bowler, 1974), and also may affect other crayfish species, for example, *Astacus astacus* (Kossakowski, 1971; Voronin, 1971). It is widespread throughout mainland Europe (Finland: Sumari and Westman, 1969; France: Vey and Vego, 1972; Germany: Schäperclaus, 1954; Poland: Kossakowski, 1971; Russia: Voronin, 1971) and Britain (Cossins, 1972), and has been reported in varying intensities. In Finland it was reported at a frequency of less than 1% (Sumari and Westman, 1969) whilst in Germany it caused great damage to stocks and occurred at a frequency of over 30% in some waterways (Schäperclaus, 1954). In Britain it has been reported at a frequency of 18% and has been implicated in population fluctuations (Pixell-Goodrich, 1956). By contrast, other populations have been known to support a relatively low frequency of the disease without apparent harm (e.g. less than 10% over several years in a Northumberland aqueduct, Cossins, 1972; Brown, 1979).

The parasite has only one host, the crayfish, and is probably spread by ingestion of parasitized muscle tissue when dead or dying animals are cannibalized (Johnson, 1977). The parasite then invades the body through the stomach wall and affects striated muscle blocks, even including those of the eye-stalks (Cossins, 1972). It is only when the disease is well established that it may easily be detected due to the white nature of the abdominal muscle (see Plate 2.1) resulting from widespread spore infestation. The effect of the disease is to cause a deterioration of muscle function leading ultimately to death, and it has a time span of about one year (Brown, 1979).

Markfield Quarry was found to be totally free of *T. contejeanii* whilst in Nanpantan Reservoir the level of infestation was very low. Higher levels were reported from the Leen population and almost a five-fold increase in the frequency of the disease occurred from 1980 to 1981. It is suggested that this may be one reason to explain the decreased population density in the second year, although the levels did not reach plague proportions. All sizes of the population from 13 mm to 45 mm (C.L.) were found to be susceptible to infestation, and no differences were observed between the sexes. A similar pattern of infestation was reported for *A. pallipes* in Northumberland, and the overall frequency of the disease in that population was similar to that seen in the Leen during 1981 (6.57% in Northumberland cf. 6.20% in 1981, River Leen). Holdich *et. al.*, (1978) report that the level of infestation in Midlands populations is 9%, but do not identify the population. The levels in all the populations studied by this author are less than this value, so it seems that some variation in the frequency of the disease may naturally occur. However, it is maintained at a sufficiently low level that a sympatric relationship may exist between the host and parasite without decimating the host population.

TABLE 4.4 THE RESULTS OF ANALYSIS OF EACH MONTHLY COLLECTION BY POLYMODAL SIZE FREQUENCY ANALYSIS FOR THE RIVER LEEN MALES TO SHOW THE SIZE STRUCTURE OF THE POPULATION THROUGHOUT THE YEARS 1980 AND 1981

N.B. The total number 'N' used in the analysis may be greater than that indicated in Fig. 4.1 which relates to the monthly collections. Here, in order to obtain a larger sample size for analysis, the mark-recapture data for corresponding months has been pooled. This also applies to Table 4.5.

MONTH	1980				1981			
	YEAR CLASS	NO. IN YEAR CLASS	% OF POPn.	TOTAL N	YEAR CLASS	NO. IN YEAR CLASS	% OF POPn.	TOTAL N
WINTER (DEC-MAY)	0+	28	28.28	99	0+	12	9.38	128
	1+	44	44.44		1+	71	55.47	
	2+	19	19.19		2+	24	18.75	
	3+	6	6.06		3+	18	14.63	
	4+	2	2.02		4+	3	2.34	
JUNE	0+	8	19.04	42	0+	0	0	30
	1+	12	28.57		1+	14	46.7	
	2+	5	11.90		2+	7	23.3	
	3+	11	26.19		3+	5	16.7	
	4+	4	9.52		4+	4	13.3	
	5+	2	4.76					
JULY	0+	106	63.47	167	0+	125	65.45	191
	1+	18	10.78		1+	6	3.14	
	2+	19	11.38		2+	33	17.28	
	3+	12	7.19		3+	19	9.95	
	4+	6	3.59		4+	7	3.67	
	5+	6	3.59		5+	1	0.52	
AUGUST	0+	26	13.51	74	0+	48	43.64	110
	1+	7	9.50		1+	6	5.45	
	2+	(16)	(21.62)		2+	35	31.82	
	3+	(12)	(16.22)		3+	10	9.09	
	4+	(10)	(13.51)		4+	6	5.45	
	5+	(3)	(4.05)		5+	5	4.55	
SEPTEMBER	0+	14	22.95	61	0+	17	43.59	39
	1+	8	13.11		1+	5	12.82	
	2+	22	36.07		2+	9	23.08	
	3+	7	11.47		3+	8	2.05	
	4+	7	11.47					
	5+	3	4.92					
OCTOBER	0+	8	21.62	37	0+	41	65.08	63
	1+	12	32.43		1+	4	6.35	
	2+	12	32.43		2+	4	6.35	
	3+	4	10.81		3+	14	22.22	
	4+	1	2.70					
NOVEMBER	0+	5	7.04	71	0+	11	57.89	19
	1+	24	33.80		1+	2	10.53	
	2+	30	42.25		2+	4	21.05	
	3+	10	14.08		3+	2	10.53	
	4+	2	2.82					

TABLE 4.5 THE RESULTS OF ANALYSIS OF EACH MONTHLY COLLECTION BY POLYMODAL SIZE FREQUENCY ANALYSIS FOR THE RIVER LEEN FEMALES TO SHOW THE SIZE STRUCTURE OF THE POPULATION THROUGHOUT THE YEARS 1980 AND 1981

MONTH	1980				1981			
	YEAR CLASS	NO. IN YEAR CLASS	% OF POPn.	TOTAL N	YEAR CLASS	NO. IN YEAR CLASS	% OF POPn.	TOTAL N
WINTER (DEC-MAY)	0+	21	22.34	94	0+	9	16.07	56
	1+	37	39.36		1+	25	44.64	
	2+	26	27.66		2+	8	14.29	
	3+	7	7.45		3+	10	17.86	
	4+	3	3.19		4+	4	7.14	
JUNE	0+	8	15.4	52	0+	-	-	15
	1+	10	19.2		1+	3	20	
	2+	5	9.62		2+	3	20	
	3+	11	21.15		3+	6	40	
	4+	12	23.08		4+	3	20	
	5+	6	11.54					
JULY	0+	106	53.81	197	0+	125	59.52	210
	1+	14	7.11		1+	12	5.71	
	2+	24	12.18		2+	18	8.57	
	3+	20	10.15		3+	19	9.05	
	4+	23	11.68		4+	36	17.14	
	5+	10	5.22					
AUGUST	0+	26	35.62	73	0+	48	35.56	135
	1+	13	17.81		1+	9	6.67	
	2+	13	17.81		2+	36	26.67	
	3+	16	21.92		3+	23	17.04	
	4+	5	6.85		4+	19	14.07	
SEPTEMBER	0+	15	20.83	72	0+	17	34.69	49
	1+	9	12.5		1+	5	10.20	
	2+	17	23.61		2+	12	24.49	
	3+	22	30.56		3+	15	30.61	
	4+	9	12.50					
OCTOBER	0+	8	19.51	41	0+	22	51.16	43
	1+	8	19.51		1+	4	9.30	
	2+	12	29.27		2+	5	11.63	
	3+	11	26.83		3+	4	9.30	
	4+	2	4.88		4+	8	18.60	
NOVEMBER	0+	1	2.33	43	0+	8	40	20
	1+	18	41.86		1+	7	35	
	2+	16	37.21		2+	3	15	
	3+	8	18.60		3+	2	10	

TABLE 4.6 AN ANALYSIS OF VARIOUS SUBPOPULATIONS OF THE RIVER LEEN AND NANPANTAN RESERVOIR POPULATIONS TO DESCRIBE THE INCIDENCE OF THE DISEASE *THELOHANIA CONTEJEANII* HENNEGUY

POPULATION	YEAR	SUBPOPULATION EXAMINED (SIZES = C.L., mm)	TOTAL NUMBER EXAMINED	NUMBER DISEASED	PROPORTION DISEASED (%)	SIZE RANGE OF DISEASED ANIMALS (mm C.L.)
RIVER LEEN	1980	WHOLE POPn. >10 mm	611	8	1.3	16 - 31 mm
		M + F ≥25 mm	288	5	1.7	
		M + F <25 mm	323	3	0.9	
	1980	ALL MALES >10 mm	294	2	0.7	19 - 27 mm
		M ≥25 mm	136	1	0.7	
		M <25 mm	157	1	0.6	
	1980	ALL FEMALES >10 mm	318	6	1.9	16 - 31 mm
		F ≥25 mm	152	4	2.6	
		F <25 mm	166	2	1.2	
	1981	WHOLE POPn. >10 mm	420	26	6.1	13 - 45 mm
		M + F ≥25 mm	192	18	9.4	
		M + F <25 mm	228	8	3.5	
	1981	ALL MALES >10 mm	206	13	6.3	15 - 45 mm
		M ≥25 mm	95	11	11.6	
		M <25 mm	111	2	1.8	
	1981	ALL FEMALES >10 mm	214	13	6.1	13 - 36 mm
		F ≥25 mm	97	7	7.2	
		F <25 mm	117	6	5.1	
NANPANTAN RESERVOIR	1979	WHOLE POPn. >10 mm	762	6	0.79	22.1 - 37 mm
		M + F ≥25 mm	550	5	0.91	
		M + F <25 mm	212	1	0.47	
	1979	ALL MALES >10 mm	389	4	1.03	
		M ≥25 mm	279	3	1.08	
		M <25 mm	110	1	0.91	
	1979	ALL FEMALES >10 mm	373	2	0.54	
		F ≥25 mm	271	2	0.74	
		F <25 mm	102	0	0.00	

TABLE 4.7 AN ANALYSIS OF VARIOUS SUBPOPULATIONS OF THE RIVER LEEN AND MARKFIELD QUARRY POPULATIONS TO DESCRIBE THE INCIDENCE OF VARIOUS TYPES OF SKELETAL DAMAGE

POPULATION	YEAR	SUBPOPULATION EXAMINED (SIZES = C.L. (MM))	TOTAL NUMBER EXAMINED	NUMBER DAMAGED	PROPORTION DAMAGED (%)	TYPE OF DAMAGE EXPRESSED AS A PROPORTION(%) OF THE TOTAL NUMBER DAMAGED							
						1	2	3	4	5	6	7	8
RIVER LEEN	1980	WHOLE POPn. >10 mm	611	91	14.89	71.4	19.8	1.1		2.2		1.1	4.4
		M + F ≥25 mm	288	35	12.15	77.1	11.4	2.9		2.9			5.7
		M + F <25 mm	323	56	17.34	67.9	25.0			1.8		1.8	3.6
	1980	ALL MALES >10 mm	294	37	12.63	67.6	27.0	2.7					2.7
		M ≥25 mm	136	16	11.76								
		M <25 mm	157	21	13.38								
	1980	ALL FEMALES >10 mm	318	51	16.04	73.1	13.5			3.80		1.90	5.80
		F ≥25 mm	152	19	12.50								
		F <25 mm	166	32	19.27								
	1981	WHOLE POPn. >10 mm	420	112	26.67	69.6	16.1	5.4	0.9	2.7		2.7	2.7
		M + F ≥25 mm	192	55	28.64	74.5	14.5	3.6	1.8	1.8		1.8	1.8
		M + F <25 mm	228	57	25.00	64.9	17.5	7.0		3.5		3.5	3.5
	1981	ALL MALES >10 mm	206	57	27.67	71.9	17.5	3.5		3.5		1.8	1.8
		M ≥25 mm	95	30	31.58								
		M <25 mm	111	27	24.32								
	1981	ALL FEMALES >10 mm	214	53	24.77	67.9	13.2	7.5	1.9	1.9		3.8	3.8
		F ≥25 mm	97	33	34.02								
		F <25 mm	117	20	17.09								
MARKFIELD QUARRY	1979	WHOLE POPn. >10 mm	412	45	10.90	86.7	6.7			2.2	2.2	4.4	
		M + F ≥25 mm	381	43	11.30								
		M + F <25 mm	31	2	6.50								
	1979	ALL MALES >10 mm	239	21	8.80	76.2	9.5			4.8	4.8	4.8	
		M ≥25 mm	228	18	8.30								
		M <25 mm	11	2	18.20								
	1979	ALL FEMALES >10 mm	173	24	13.90	92.0	4.0				4.0		
		F ≥25 mm	153	24	15.70								
		F <25 mm	20	0	0.00								

TYPES OF DAMAGE

1. One chela missing, damaged or regenerating.
2. Both chelae missing, damaged or regenerating.
3. Rostrum damaged.
4. Telson damaged.
5. Carapace damaged.
6. Abdomen damaged.
7. More than one of 1-6.
8. Damaged by net when caught.

FIGS. 4.1 - 4.4

Figs. 4.1 and 4.3 show the seasonal variation in catch size experienced at the Leen and Markfield population study sites respectively. Some of the variation may be explained by recruitment (the shaded area of Fig. 4.1 represents juveniles, <10 mm C.L.) but temperature differences throughout the year explain the major differences. Figs. 4.2 and 4.4 show the catch, expressed on a logarithmic scale, plotted against temperature, for each population. Regression analysis reveals a high degree of correlation thus;

FIG.	POPULATION	EQUATION	S.E.B.	r	F	df	P	N
4.2	LEEN	LOG. CATCH = 0.0486. TEMP. +1.0110	0.0098	0.7972	24.41	15	<0.01	17
4.4	MARKFIELD	LOG. CATCH = 0.1001. TEMP. +0.0209	0.0142	0.9207	50.05	9	<0.01	11

FIG. 4.1 TO SHOW THE SEASONAL VARIATION OF LEEN CATCH SIZES.
(THE SHADED AREA REPRESENTS JUVENILES)

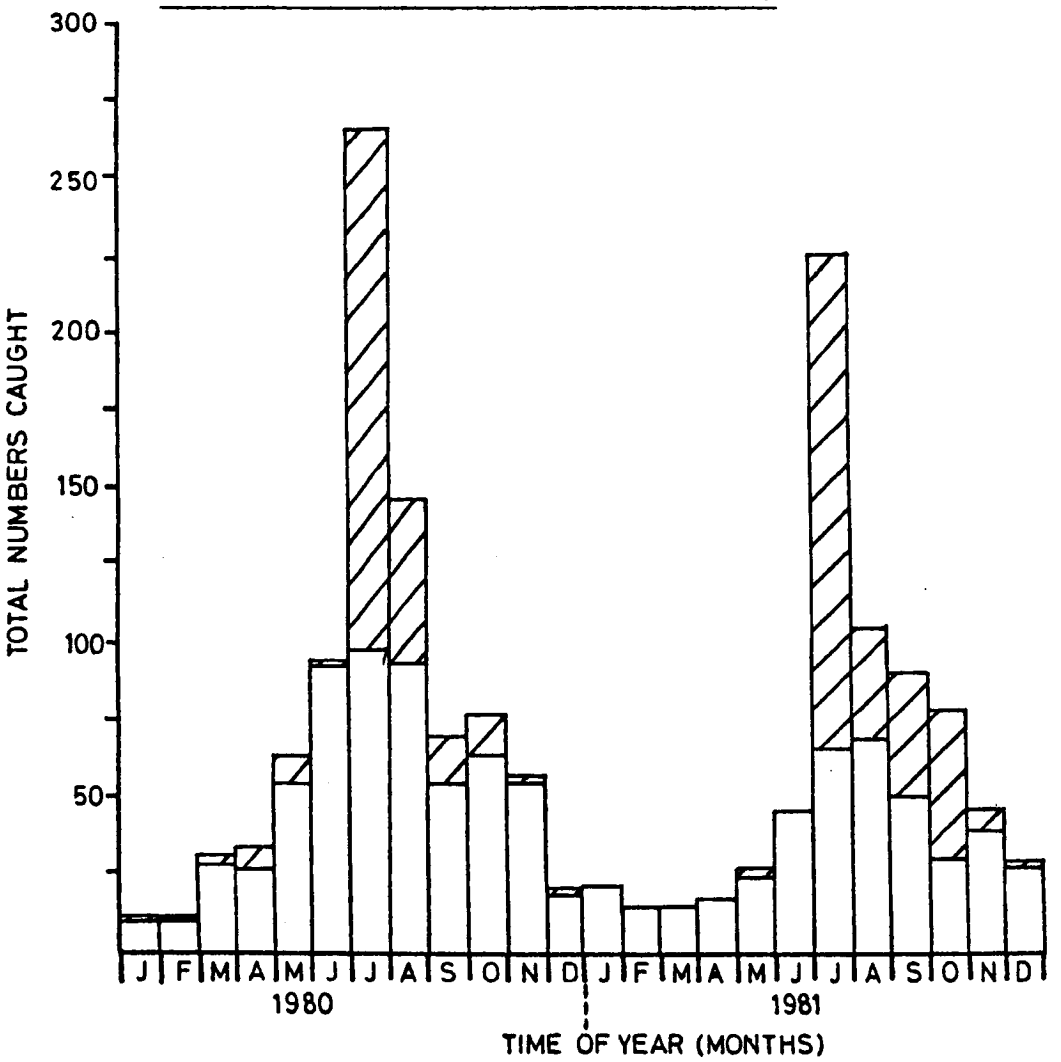


FIG. 4.2 TO SHOW LOG. ADULT CATCH AGAINST TEMPERATURE FOR THE LEEN POPULATION

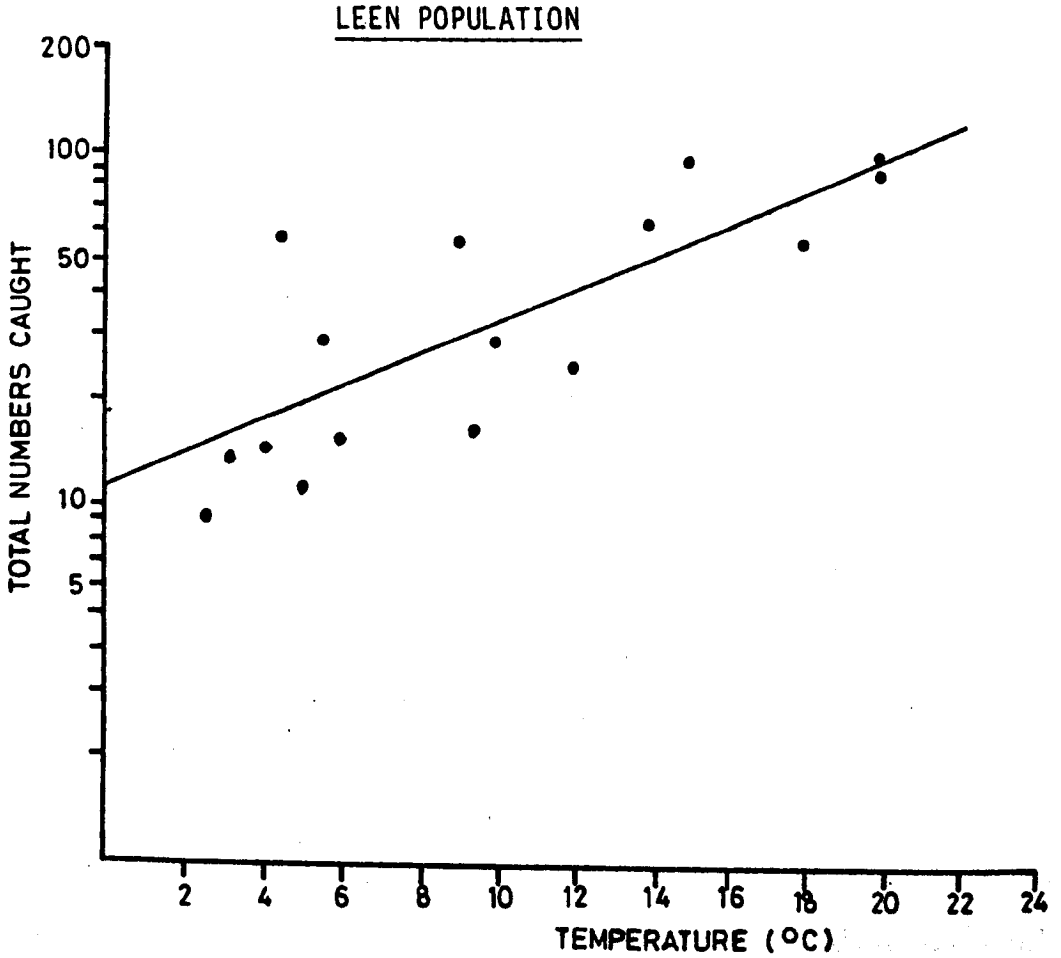


FIG. 4.3 TO SHOW THE SEASONAL VARIATION OF MARKFIELD CATCH SIZES (1981) BY TRAPPING

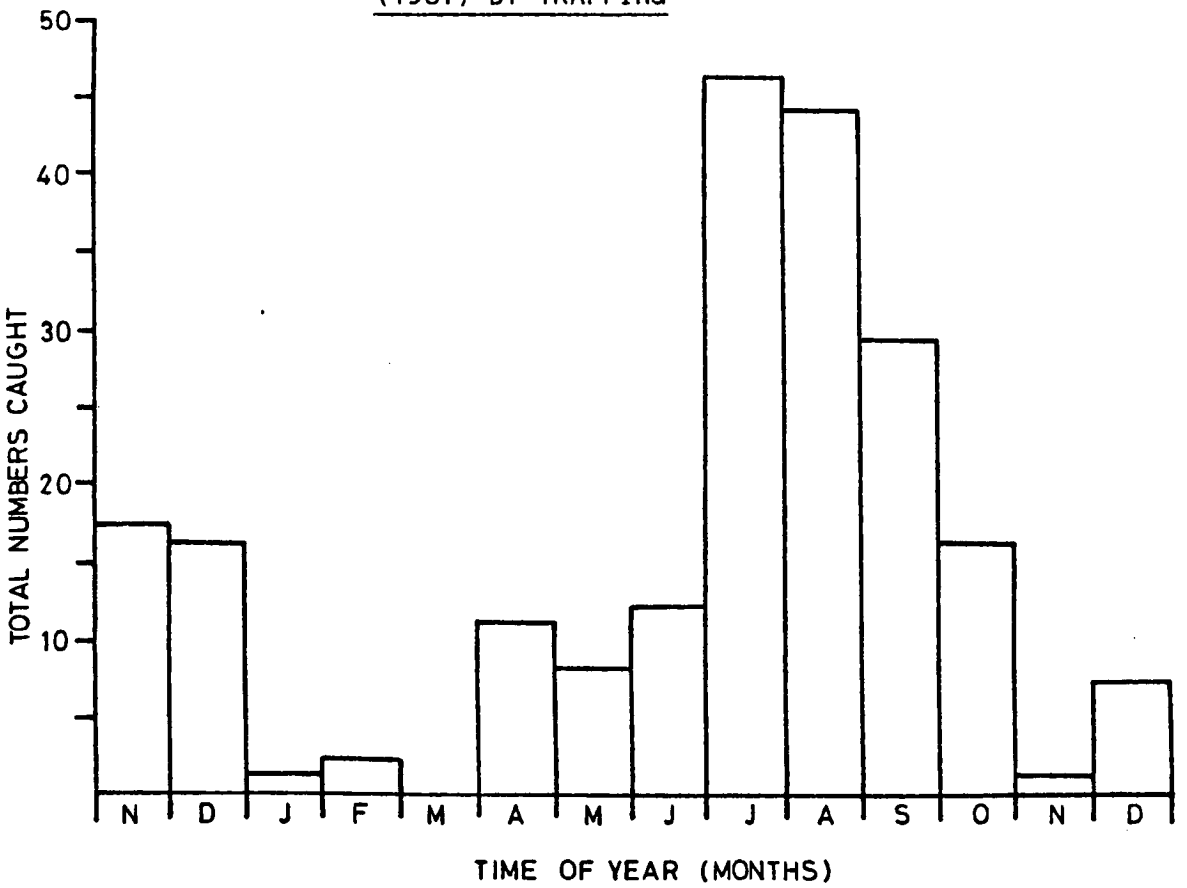
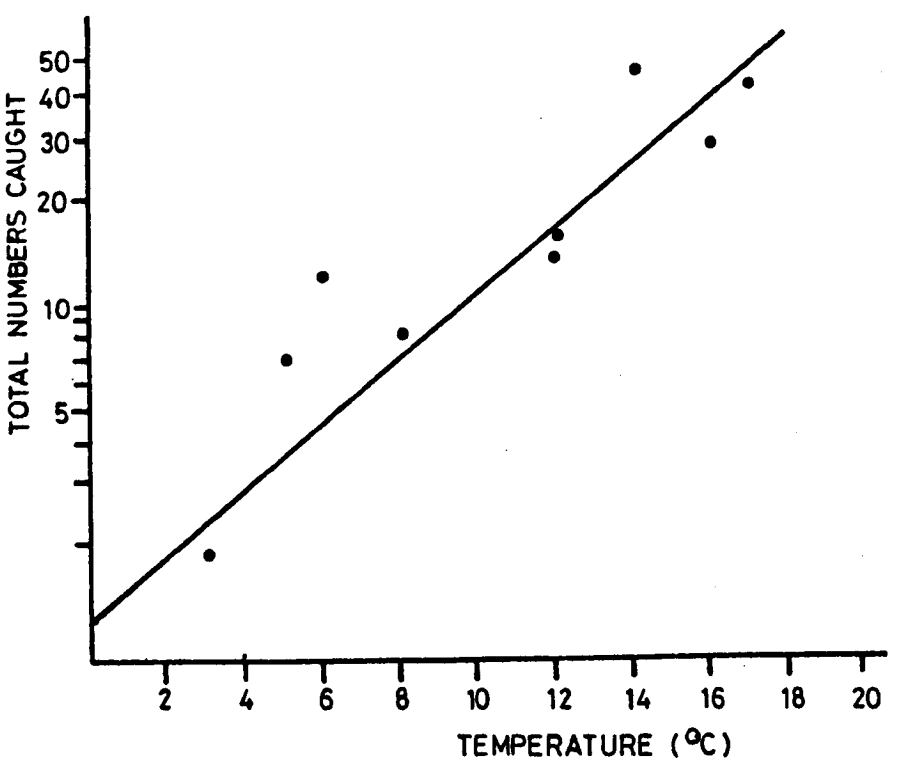


FIG. 4.4 TO SHOW LOG. CATCH AGAINST TEMPERATURE FOR THE MARKFIELD POPULATION



FIGS. 4.5 - 4.10

These figures show the seasonal variation of certain parameters throughout the study period. They illustrate the population structure over this period.

Figs. 4.5 - 4.7 relate to the Leen population and show; the proportion of juveniles (< 10 mm C.L.) represented in the monthly catches (4.5), and the sex ratio (< 1 indicates an excess of females to males, >1 indicates the opposite) of mature animals (>25 mm C.L., 4.6) and immature animals (<25 mm, 4.7). 4.8 indicates the sex ratios observed for the total Markfield population as collected by snorkelling, and trapping. The solid lines between arrows in Figs. 4.6 - 4.8 relate to the period when females may be expected to be carrying eggs.

Figs. 4.9 and 4.10 show the proportion of mature animals (> 25 mm C.L.) represented in the River Leen monthly catches for males (4.9) and females (4.10).

Fig. 4.5

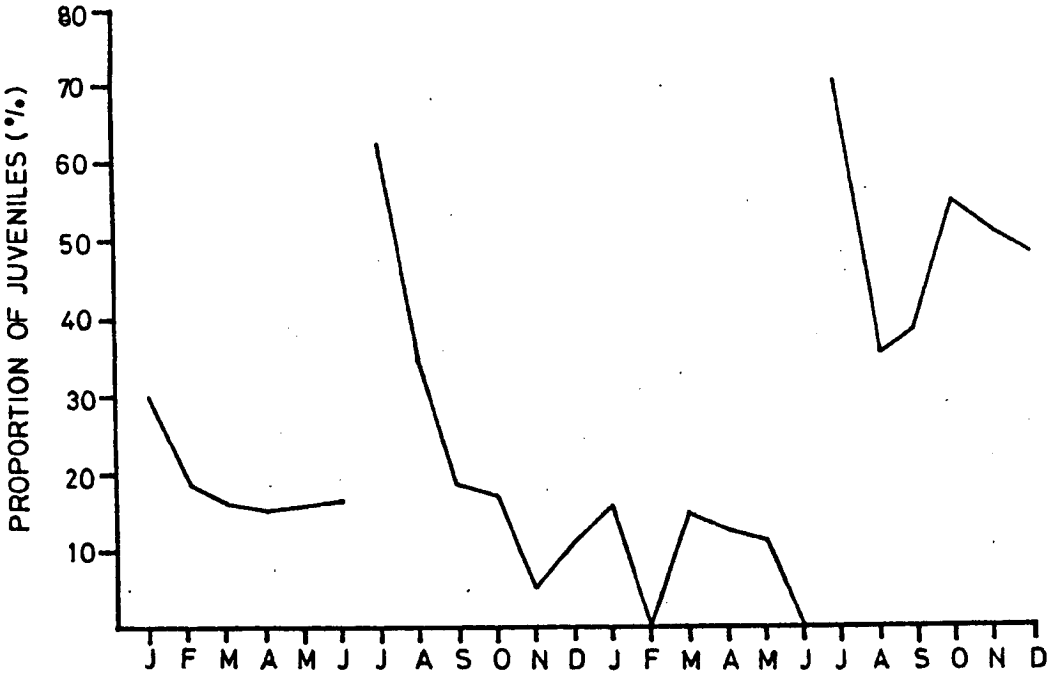


Fig. 4.6

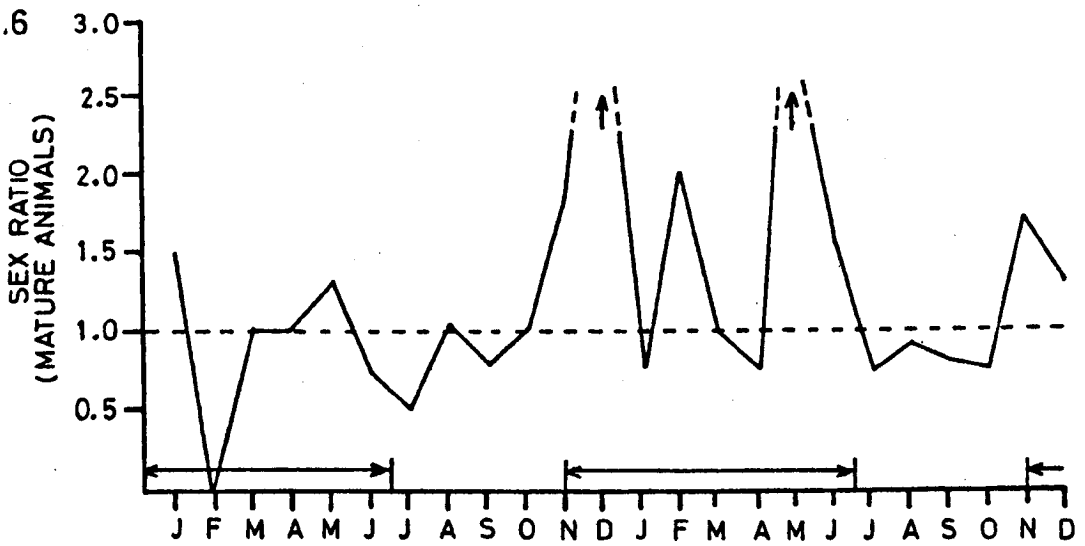


Fig. 4.7

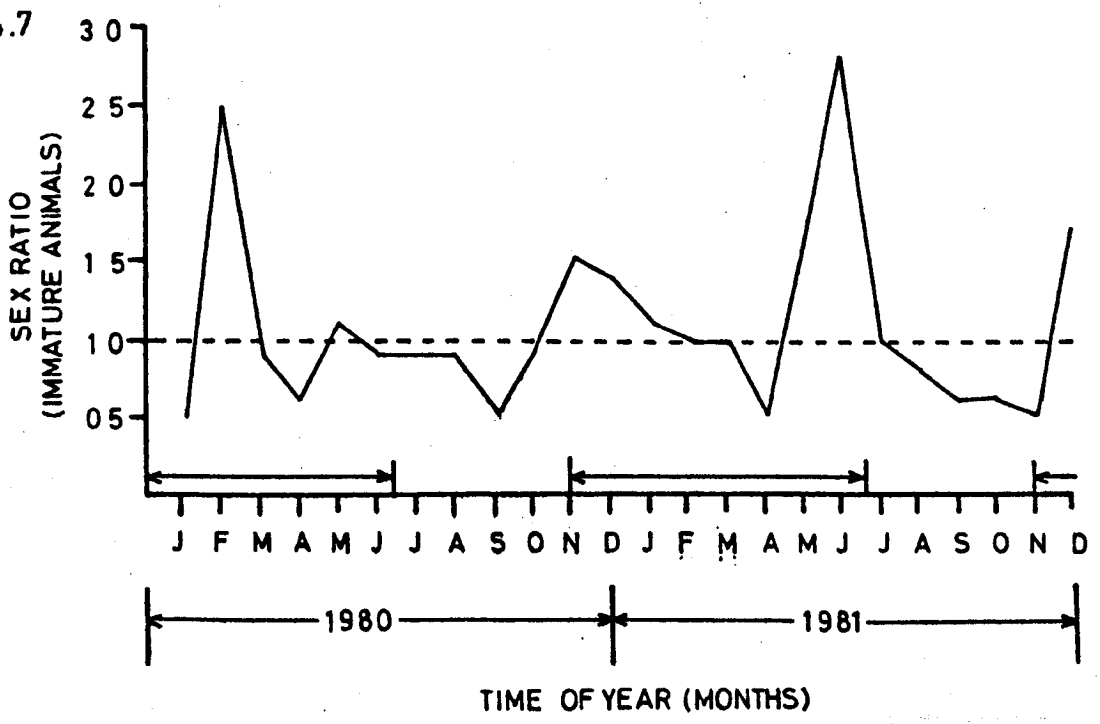


Fig 4.8

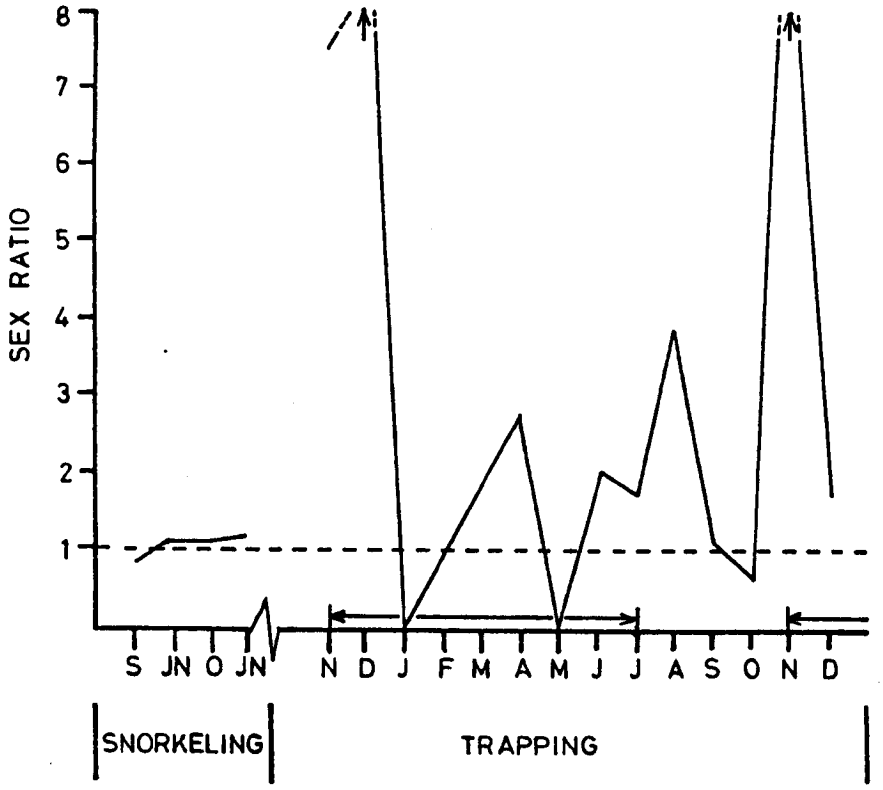


Fig. 4.9

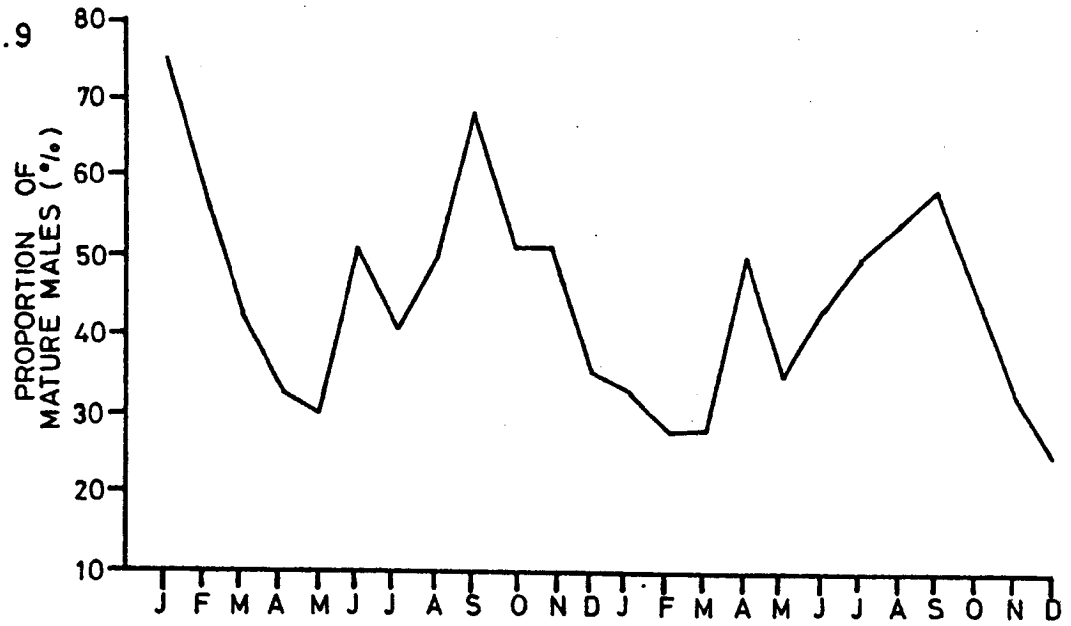


Fig.4.10

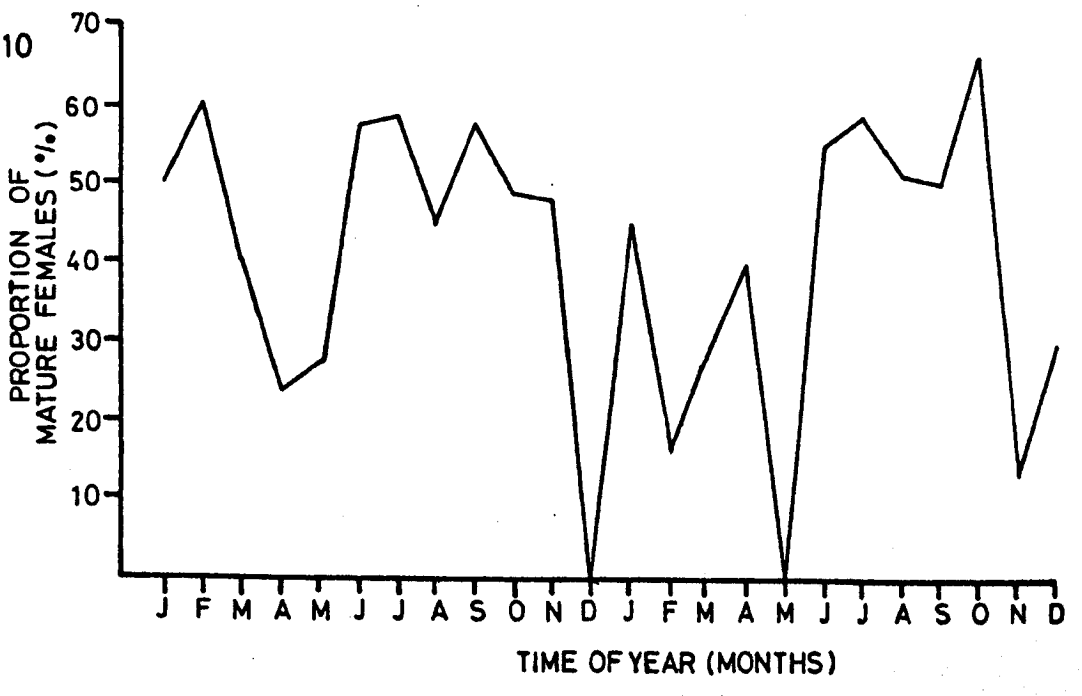


FIG. 4.11 TO SHOW THE SIZE-FREQUENCY DISTRIBUTION OF DISEASED LEEN CRAYFISH

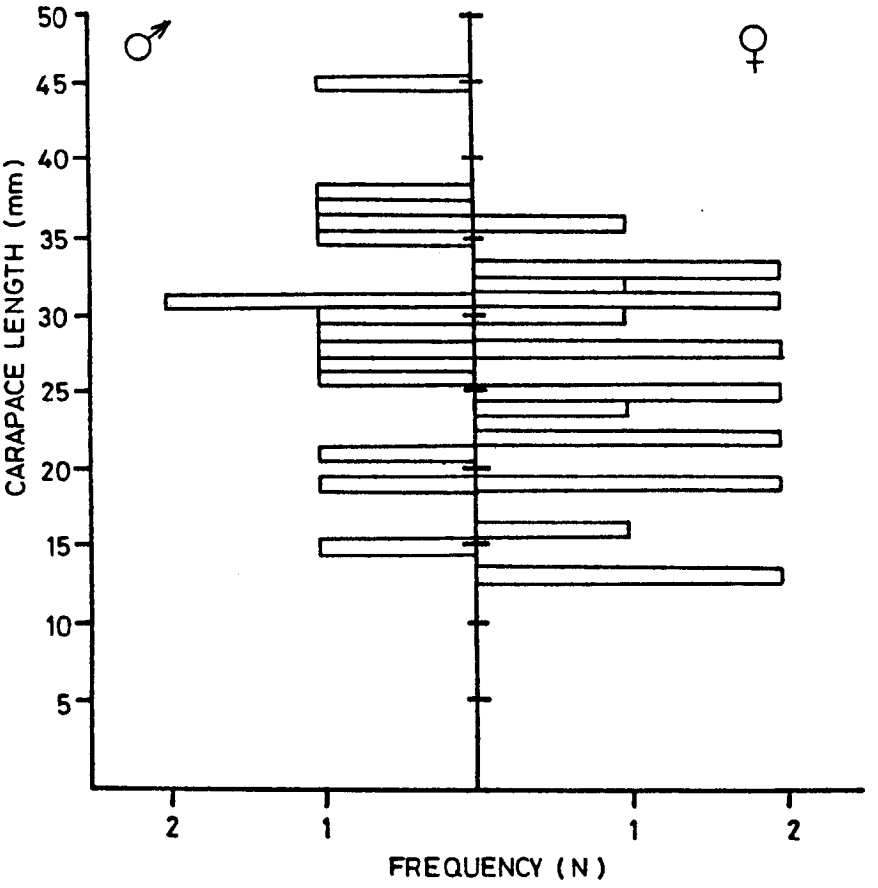


FIG. 4.12 TO SHOW THE SIZE-FREQUENCY DISTRIBUTION OF DAMAGED LEEN CRAYFISH

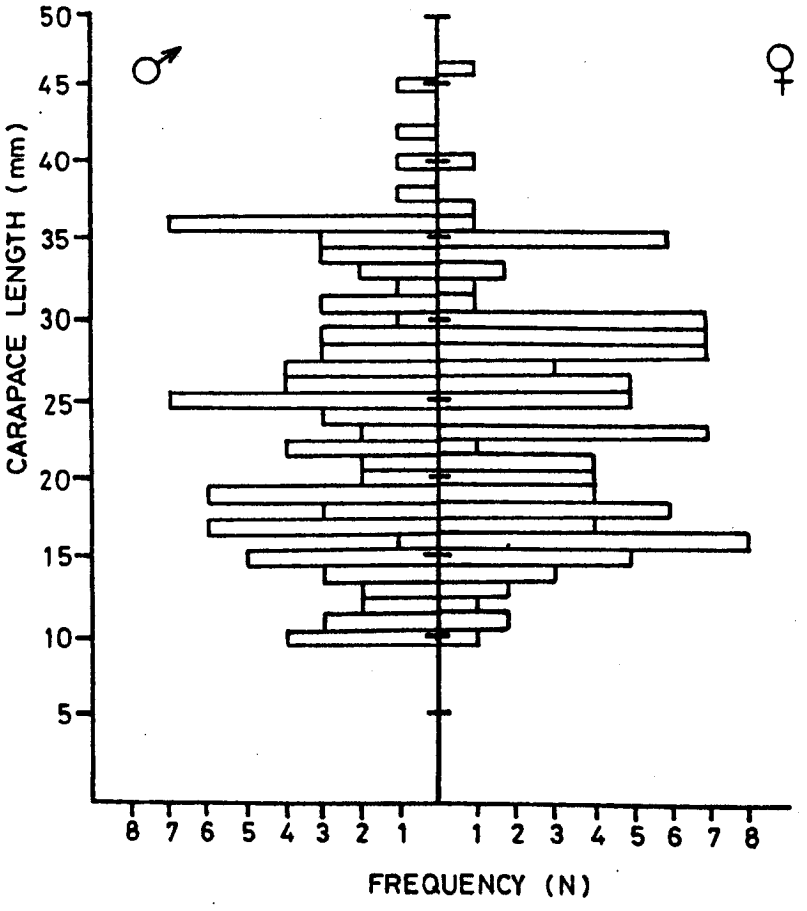


FIG. 4.13 TO SHOW THE AMOUNT OF LIVER CONSUMED BY OVIGEROUS (—) AND NON-OVIGEROUS (---) FEMALE CRAYFISH DURING FEEDING TRIALS

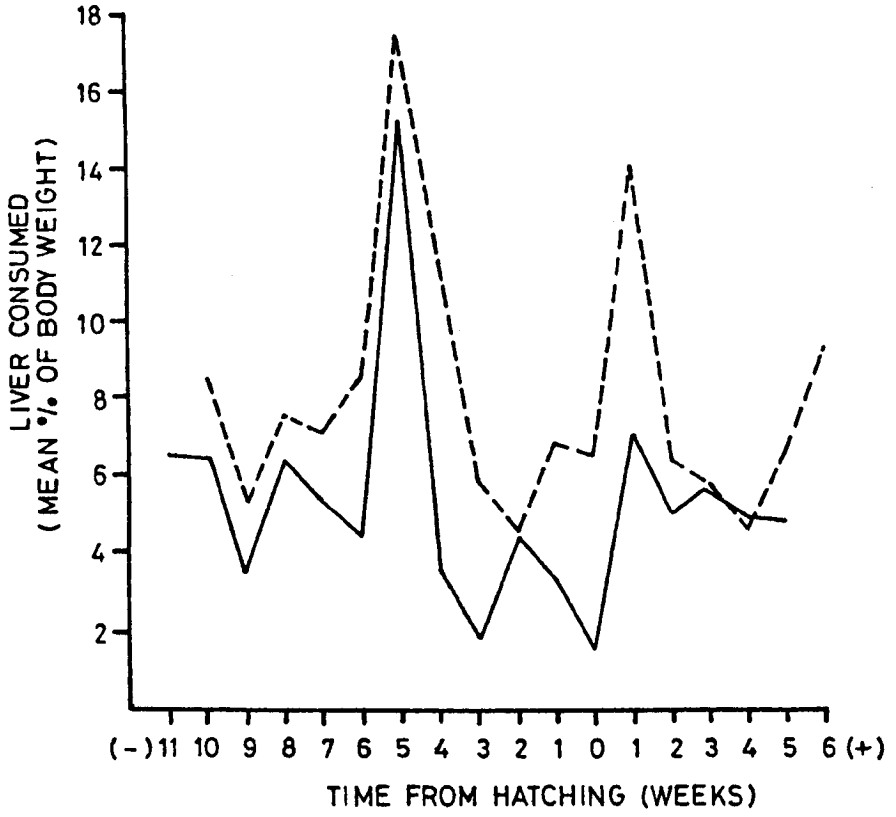


FIG. 4.14 TO SHOW THE PROPORTION OF OVIGEROUS (—) AND NON-OVIGEROUS (---) FEMALE CRAYFISH NOT FEEDING DURING FEEDING TRIALS

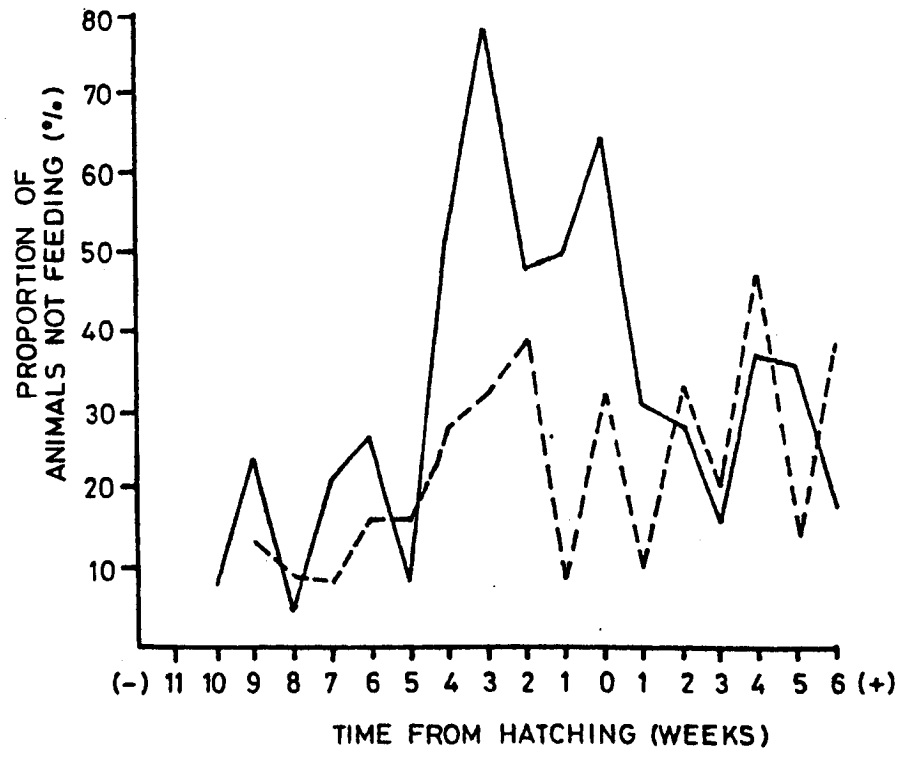
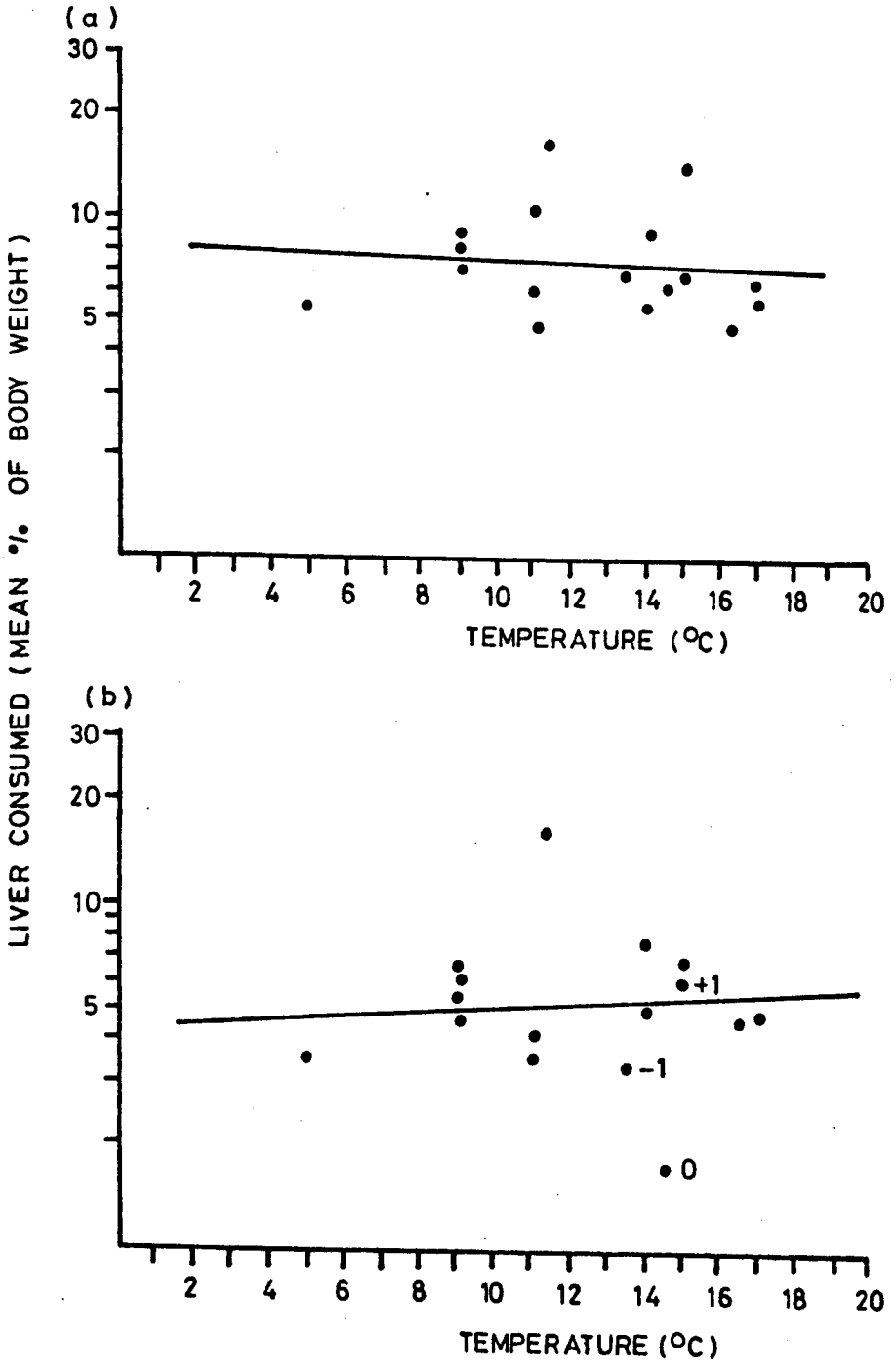


FIG. 4.15 TO SHOW THE AMOUNT OF LIVER CONSUMED BY CRAYFISH DURING FEEDING TRIALS EXPRESSED ON A LOGARITHMIC SCALE, PLOTTED AGAINST TEMPERATURE FOR a) OVIGEROUS FEMALES, b) NON-OVIGEROUS FEMALES (THE LINES FITTED TO THE POINTS SHOW NO CORRELATION BETWEEN TEMPERATURE AND THE AMOUNT OF FOOD CONSUMED see 4.2)



CHAPTER 5

STUDIES ON THE GROWTH, RELATIVE GROWTH, AND MORPHOLOGY OF *A. PALLIPES*

5.1 GROWTH OF *A. PALLIPES* IN THE POPULATION STUDY AREAS

5.1(i) INTRODUCTION

In Britain, it has been suggested that we should "explore the native potential first" (Goddard and Holdich, 1979). This is a reference to the introduction of foreign crayfish species into Britain for culture purposes, aimed at satisfying the luxury food market. The suggestion is that *A. pallipes* could form a viable alternative. The meat yield of this species has been studied, and was found to compare favourably with other species of crayfish. Growth under laboratory conditions has also been described for the juvenile stages of the life cycle (Rhodes, 1980). However, it is the growth rate of natural populations which requires further consideration, since it is these that we may wish to exploit. It is necessary then, to compare such findings with data relating to the growth rates of the non-indigenous crayfish species, and to determine factors such as the time required to reach a marketable size.

Growth in the Crustacea is achieved by moulting. The process of cell division and the growth of the soft tissues is a continuous process (see Brown, 1979, for review). Described in linear and gravimetric terms, however, growth is seen to be discontinuous, occurring in a stepwise fashion, apart from some slight stretching of the integument which may occur between moults. This causes a slight increase in linear dimensions, as has been reported for the shrimp, *Crangon crangon* and several species of mysids (Mauchline, 1973).

The study of crustacean growth involves attempts to describe the nature of the "steps", and considers both the moult increment, (i.e. increase in size at moulting) and the moult frequency. Field studies have often employed indirect methods to estimate both of these factors, due to the difficulties of obtaining sufficient recaptures which would enable their direct assessment. In this study accurate observations of both moult increment and frequency have been possible due to the mark and recapture programme which enabled the monitoring of individual crayfish throughout periods of their life history. Thus it has been possible to form an accurate assessment of the pattern of growth.

Large variations in both moult increment and moult frequency have been observed to occur, (see 5.1(iii)). They may occur both between individuals, and within the same individual (Brewis and Bowler, 1982). Furthermore, the evidence also suggests that these factors are age or size dependent. Expressed relative to body size, the moult increment is seen to decrease with increasing size/age (e.g. Hopkins, 1967). Moult frequency has also been observed to decrease with age (e.g. Hopkins, 1967; see also 5.1(iii)).

Decapod crustaceans possess no external markings which may indicate their age, and thus aid growth studies. At each moult the old exoskeleton is shed leaving no record of previous moults. It has been found possible to age Isopods by assessing the number of aesthetascs on the antennules (Holdich, 1968), but no similar correlations have been found applicable to the decapod Crustacea (Farmer, 1973; Brown, 1979). It has hence been necessary to employ indirect methods which relate the size of animals to their age

(e.g. Kurata, 1962; Hopkins, 1967; Abrahamsson, 1971; Brown and Bowler, 1978). The method involves separating out modal size classes by size frequency analysis, (Harding, 1949; Cassie, 1954) and then substantiating that each mode represents a year class by considering also moult increment and moult frequency data.

In addition to the age dependent differences observed in the growth rates of decapod Crustacea, differential rates have also been reported to occur between the sexes (see 5.1(iii)). Further complications may arise where an individual is regenerating a chela (e.g. Bennett, 1974) or is diseased, in the case of *A. pallipes* by infestation with the microsporidian parasite. *Thelohania contejeani* Henneguy. Both of these result in a reduction of the absolute moult increment at ecdysis (Brown, 1979).

Thus it may be seen that the growth of decapod crustaceans requires careful and detailed analysis to properly describe it. Three methods of analysis have commonly been employed which relate to moult increment data. These are;

1. The relationship between the absolute moult increment, (in linear or gravimetric units) and the pre-moult carapace length (PrMCL).
2. The relationship between the 'growth factor', (moult increment expressed as a percentage of the PrMCL) and the PrMCL.
3. The relationship between the PrMCL and the post-moult carapace length (PoMCL).

More recently, the annual instantaneous growth rate in wet weight has been considered in relation to the PrMCL. (Pratten, 1980; Brewis and Bowler, 1982). This was not possible for the Midlands populations since insufficient recaptures were made to enable

observations of the wet weight of individuals at the beginning and end of any one year. This is necessary for the calculation of the annual instantaneous growth rate.

Hiatt (1948) was the first to employ the method of plotting PrMCL against PoMCL. By eye, he was able to fit three lines to his data, which described the growth relationship expressed by young crabs, and adult males and females. This method has subsequently been used by many authors who also describe growth data as being fitted by one or more linear regression lines, (e.g. Butler, 1961; Wilder, 1963; Forster, 1970; Farmer, 1973; Brown, 1979; Rhodes, 1980). Kurata (1962) developing this method further, postulated three types of growth coefficient, b, derived from the linear treatment of plotting the PoMCL against the PrMCL.

The general form of a linear regression line is;

$$Y = a + bX$$

'Y' is the PoMCL, 'X' the PrMCL, 'a' is a constant, and 'b' is the slope of the line, which represents the growth coefficient, the three types of which are;

(i) $b > 1.05$; Progressive geometric growth. The absolute moult increment increases with increasing PrMCL.

(ii) $b < 0.95$; Retrogressive geometric growth. The absolute moult increment decreases with increasing PrMCL.

(iii) $b > 0.95, <1.05$; Arithmetic growth. The absolute moult increment remains constant with increasing PrMCL.

This argument has been taken further still. Since the values of 0.95 aand 1.05 were chosen arbitrarily, they will not take into account sample variation. The parameters defining geometric growth should therefore not be fixed at these values, and growth

may only be argued to differ from arithmetic growth if the value of 'b' is observed to differ significantly from unity in either direction, (Farmer, 1973; Brown, 1979).

Thus it may be seen that the practice of fitting linear regression lines to data of type 3, above, has been commonly employed and enlarged upon. These lines are referred to as Hiatt growth diagrams. However, it will be seen that in most cases this is incorrect. For the Hiatt growth diagram to be described in linear terms, the growth factor must either be constant, or else change at a constant rate. In most cases it does not, (see 5.1(iii)) and the relative decrease in the growth factor which is observed tends to increase with increasing size. This would implicate a curve for the Hiatt growth diagram. Both Hopkins (1967) and Mauchline (1976) share this view. The former suggests a curvilinear relationship between the PoMCL and the PrMCL, with the PoMCL being proportional to the square of the PrMCL. Mauchline (1976) argues that the decrease in growth factor is usually logarithmic against PrMCL which would result in the form of Hiatt growth diagram being that of a hyperbola. He also states that since the relationship of PoMCL to PrMCL is that of a curve, "the derivation of growth constants through linear regression analysis is not correct". A method for calculating the equation of the hyperbola is given.

There have now been a large number of studies conducted which relate to the growth of decapod crustaceans, both in field and laboratory situations (see 5.1(iii)). Most employ one or more of the three methods described for moult increment data, but only those mentioned above treat the Hiatt growth diagram

as a curve. Since it is felt by this author, that a curve is the correct interpretation of growth data of this type, the methodology in the analysis of the results of this study is based upon that hypothesis. The implications of such methodology are discussed (see 5.1(iii)).

Studies relating to the growth of *A. pallipes* in Britain have increased in recent years, although only two other populations have been the subject of detailed analysis. Early reports of the growth of field populations of *A. pallipes* are largely subjective and are based more on observation than analysis. These relate to a population in the River Darent, Kent (Thomas and Ingle, 1971) and a population in an Irish lake (Morriarty, 1972). Recently more rigorous studies have been conducted which employ the methods described. The populations studied were those of; a Northumberland aqueduct (Brown, 1979) in the North of England; and the River Ouse, Buckinghamshire (Pratten, 1980) in the South of England. A laboratory study of the growth of juvenile crayfish from pooled Midlands populations has been conducted (Rhodes, 1979) but growth in the laboratory has been found by several authors to be considerably less than in the field (e.g. Hiatt, 1948; see also 5.1(iii)). Thus information is wanting in relation to the growth rates of *A. pallipes* from individual Midlands populations in their natural habitat. Such details are thus presented. The two populations studied, at Markfield Quarry, and the River Leen, form geographical intermediates between those studied in the North and South of England.

5.1(ii) METHODS AND RESULTS OF ANALYSIS

MOULT INCREMENT ANALYSIS

METHODS

All animals were measured accurately with Vernier Calipers to 0.1 mm (see 2.4). The carapace length was taken as the reference dimension in all the growth studies since it affords the least errors in measurement, and is not prone to stretching of the integument which may result in errors when measuring the total length. Data relating to the absolute moult increment (MI) was gained from individually marked and recaptured animals from the River Leen. Any animals which were either diseased or regenerating a chela were not used in the analysis. For the Markfield Quarry population, since no mark and recapture study had been conducted, unmoulted animals caught in June and July of 1981 were individually marked, placed in the outdoor holding tanks, (see Section II) and then re-measured after having moulted. Again, no chelae regenerating, or diseased animals were chosen. For both populations, only animals which had moulted once since the previous measurement were used in the analysis.

The initial analyses conducted involved plotting the MI against the PrMCL and the growth factor against the PrMCL. Both linear and Log. Linear regression analyses were conducted to see which gave the best fit (Mauchline, 1976). Although linear regression proved to be the better fit for the growth factor, the results were not statistically significant, and so a Hiatt growth curve was next constructed (see 5.1(i)).

The general form of a curve is;

$$Y = BX^{\alpha}$$

which becomes;

$$\text{LOG } Y = \text{LOG } B + \alpha \text{Log } X$$

i.e., this is now the form of a linear regression line. 'B' and 'α' are constants, and 'X' represents the PrMCL whilst 'Y' represents the PoMCL. Thus regression analysis of the data is possible if Log. PoMCL is regressed against Log. PrMCL. This transformation was conducted, and the analysis was achieved with the Nottingham University ICL 2900 computer, using the SPSS package (see 2.5).

The logarithmic transformation of the data was compared against a standard linear regression analysis of the data, as has been conducted in the past. To determine which of the two models best predicts the true situation, the following must be considered;

(i) 'r'; the correlation coefficient. The best line will have the greatest correlation coefficient. A value of 1 would represent perfect correlation of the data.

(ii) 'F'; The probability that the predicted line will fit the raw data. The probability 'P' is obtained from 'F' tables (e.g. Snedecor and Cochran, 1967). The best fit line will have the greatest 'F' value.

(iii) Plots of the residuals; The SPSS package facilitates the plotting of residuals (i.e. the difference between the raw data points and the predicted line). They are plotted against zero, on the abscissa, which represents the predicted line. Deviation should be even about zero, and any positive or negative trends indicate that the true line deviates from the predicted line (see 5.2 for example).

The method of fitting a hyperbola to the data was also attempted (Mauchline, 1976) but the results gave a predicted line which was so obviously incorrect as to suggest that there may have been a printing error in the description of the equations? Mauchline (1976) states that the comparison of hyperbolae is rather cumbersome. The method described above results in the expression of the Hiatt diagram in linear terms, and thus it is a simple matter to compare different sets of data by comparing the slopes of the lines predicted. The t-test may be used, where 't' is calculated thus;

$$t = \frac{b_1 - b_2}{\sqrt{\frac{df_1 SE_1^2 + df_2 SE_2^2}{df_1 + df_2}}}$$

The degrees of freedom for 't' are;

$$df = \frac{1}{\frac{U_2}{n_1 - 2} + \frac{(1-U)^2}{n_2 - 2}}$$

where,

$$u = \frac{(SE_{biggest})^2}{SE_1^2 + SE_2^2}$$

'b_1', and 'b_2' are the slopes of the two lines.

'SE_1' and 'SE_2' are the Standard errors of 'b_1' and 'b_2' respectively.

'df_1' and 'df_2' are the degrees of freedom for the two lines.

'N_1' and 'N_2' are the number of data points on each line.

RESULTS

From the River Leen population, a total of 49 males and

51 females which had been marked were observed to have moulted on subsequent recapture. Of this number, only 28 males and 34 females fitted the criteria of having moulted only once between captures, and of being disease and damage free, thus enabling their use in moult increment analysis. 22 males and 15 females from the Markfield population were used. Unfortunately these were all mature animals due to the earlier moulting of juveniles, and also the tendency to catch adults rather than juveniles in the traps (see 2.1(iii)).

The initial analyses conducted, in which MI and growth factor were related to PrMCL, proved largely to be statistically insignificant, thus precluding any definitive statements. This was due to the relatively small sample sizes involved, and the considerable variability between moult increments which was observed. This variability, which occurred at all sizes of PrMCL, is illustrated in Figs. 5.1, a+d. Table 5.1 shows a comparison of the linear and log. linear regression analyses of the data. Excepting the Markfield females, the best fit is given by the log. linear transformation of the data, which indicates that it is best described by a curve. With increasing PrMCL the MI is not constant.

Only the analysis of the Leen males produced a statistically significant result ($P < 0.05 > 0.025$). All the results were analysed further by comparing the slope of the regression against zero. For both male and female subpopulations from the River Leen a significant deviation occurred in a positive direction ($P < 0.05 > 0.025$) which indicates that for increasing size (PrMCL), the absolute moult increment also increased. Neither the Markfield

males nor females differed significantly from zero ($P > 0.5$ and > 0.2 respectively). Thus no change is observed to occur in the MI with increasing size.

Similar analyses were conducted for the growth factor. The results are expressed in Table 5.2 and figures 5.2 a→d. In each case a linear description of the data describes the most accurate fit, but only the Leen female subpopulation gives a statistically significant result ($P < 0.01$). All have a negative slope which indicates that the growth factor decreases with increasing size, but this assertion only proves to be statistically valid for the Leen females where a significant deviation from zero is observed to occur, ($P < 0.005 > 0.001$)..

These initial analyses, supported by more definitive data in the literature (e.g. Hiatt, 1948; Kurata, 1962; Mauchline, 1976; Pratten, 1980; Brewis and Bowler, 1982) formed the basis of the hypothesis that the Hiatt growth diagram should be represented by a curve. The following describes the analysis of the relationship between the PrMCL and the PoMCL based upon this assertion.

Table 5.3 compares the linear analysis of the data with that of the log. transformed data. The results are expressed in figures 5.3, a→d. With the exception of the Markfield female subpopulation, it may be seen that the data is best described by a curve as predicted. Observation of figures 5.3, a→d reveals, however, that these curves are only very slight, whilst figures 5.4 a→d which show the log. transformed data reveal that after transformation an almost perfect straight line results. This is in accordance with excellent correlation and 'F' values obtained for the log. transformed data (see Table 5.3).

To properly define the growth of crayfish throughout the full size range it would have been necessary to have achieved more recaptures of animals at the extremes of the size range. The size range of Leen animals was 15.5 - 31.6 mm and 15.0 - 34.3 mm for males and females respectively. For Markfield animals the range was 27.3 - 38.8 mm and 28.5 - 36.5 mm for males and females respectively. This is apparent on figures 5.3, a-d, although the best fit line has been predicted from the equations in Table 5.3 and fitted to the full size range. Thus it must be stated that, as with all models, those described here strictly only relate to the data given, and that extrapolation of the model to describe the growth of juveniles of the 0+ year class, or very large adults, may not be an accurate representation of the true situation.

It will be observed from Table 5.3 that the standard linear regression analysis of the data also produces a highly significant correlation between PrMCL and PoMCL, (and indeed, best describes the Markfield female subpopulation). However, this analysis does not fully describe the true situation occurring. Thus, comparisons between subpopulations were made using the log. transformed data, and this applied also to the females of the Markfield population. Such a comparison is valid, since it will be seen that although in this instance the linear analysis best describes the data, the log. transformation also produces a highly significant correlation ($P < 0.001$), and indeed best describes all other sets of data. Comparison was made using the 't-test', described above.

It was found that no significant differences existed between

males and females for either population, (River Leen, $t = 1.8385$, $df = 46$, $P < 0.1 > 0.05$; Markfield Quarry, $t = 0.2499$, $df = 32$, $P > 0.5$). Similarly, a comparison of the Hiatt growth diagrams of the males of each population produced no significant differences, ($t = 1.7159$, $df = 30$, $P < 0.1 > 0.05$) nor did that of the females, ($t = 1.2651$, $df = 17$, $P < 0.4 > 0.2$).

In addition to the above analyses, the mean absolute moult increments of each subpopulation were also compared against each other. This value was 2.25 ±0.76 mm (N = 28), and 2.07 ±0.68 mm (N = 32) for Leen males and females respectively, and 1.68 ±0.68 mm (N = 22) and 1.21 ±0.52 mm, (N = 15) for Markfield males and females respectively. No difference was observed between the sexes of the Leen population ($t = 0.9786$, $df = 53$, $P > 0.2$) thus supporting the analysis of the Hiatt growth diagrams. Differences, however, were observed to occur between the sexes for the Markfield population ($t = 2.41$, $df = 36$, $P < 0.025 > 0.01$), the males having the greater moult increment. Comparing the two populations, differences were observed for both the sexes, the mean moult increment being significantly greater for the Leen population, (males, $t = 2.7931$, $df = 47$, $P < 0.01 > 0.005$; females $t = 4.9567$, $df = 37$, $P < 0.001$).

MOULT FREQUENCY ANALYSIS

METHODS

The annual moult frequency for crayfish of different age/size classes was derived in two ways. Direct observations of moulted, marked and recaptured animals enabled the exact numbers of moults occurring to be assessed. Animals which had clearly moulted more than once between subsequent recaptures were obvious since the

plots of these data would deviate significantly from the Hiatt growth curve. Since an extremely close relationship has been shown to exist between the PrMCL and the PoMCL it is possible to predict the number of moults which have occurred between the recaptures. Using the original measurement of carapace length on the first capture of the animal, the PoMCL is predicted from the Hiatt growth diagram. The process is repeated 'n' times until agreement is reached between the observed PoMCL and that predicted. 'n' represents the moult frequency.

The second, and indirect method of assessing annual moult frequency, is to estimate the modal sizes for each year class using polymodal size frequency analysis, (see below). Moult frequency is then predicted between the modal sizes using the Hiatt growth diagram. Since each mode is thought to represent a year class (see below) this hence provides an estimate of the annual moult frequency for animals of different ages.

RESULTS

The mark and recapture programme conducted in the River Leen enabled direct observation of moult frequency for that population. The results are expressed in table 5.4. With the exception of one female in the 16 - 18 mm size class, and one male in the 14 - 16 mm size class, which moulted five times and four times respectively, it will be seen that the maximum number of moults generally observed for any size class is three. This, however, does not mean that more than that number did not occur. Similarly, where an observation of only one moult is recorded, especially for the smaller size classes, it does not imply that only one moult occurs per annum for that size class. Such

observations occur because an animal which has been observed to have moulted at the beginning of the growth season may never have been recaptured a third time, thus precluding a definitive statement as to the annual moult frequency. Thus it is not annual moult frequency which is expressed by table 5.4. It does, however, indicate the maximum number of moults which were observed in any one year for any particular size class. It thus provides an indication of the size classes likely to complete either single or multiple summer moults.

That some crayfish in the River Leen may moult more frequently than three times a year is implied by consideration of the crayfish numbered 702, 737, 774, 842, 863, (males) and 720, 806, (females). Also, many of the smaller animals were observed to have moulted twice by the beginning of July. Since the growing season continues until the end of October, it is likely that more moults will occur in the remaining four months.

Consider animal 702. This male was first caught in October 1980. It measured 15.4 mm (carapace length) and was recorded as 'about to moult' (see 2.3). In November of the same year it was recaptured and measured 18.5 mm on this occasion. Using the equation in table 5.3 for the Leen males, two moults are predicted to have occurred, (PrMCL 15.4 → PoMCL 16.88 → PoMCL 18.47). Presumably moulting had also occurred prior to October, and so two moults is the minimum we may estimate for 1980.

In August 1981 the same animal was recaptured for a third time, now measuring 24 mm carapace length. The model predicts the following; 18.5 → 20.22 → 22.08 → 24.08 mm, i.e. three moults are required to achieve a length of 24 mm. These will all have

occurred during the 1981 growing season since November was the end of the season in 1980. With a further three months of the growing season remaining in 1981 it is possible that moulting will have occurred in excess of the three observed.

Similar arguments may be applied to the other male crayfish indicated above. Presented more briefly, these are;

NUMBER	FIRST CAUGHT	PREVIOUSLY MOULTED	CARAPACE LENGTH (mm)	RECAPTURED	CARAPACE LENGTH (mm)	PREDICTED GROWTH	NO. OF MOULTS	GROWING SEASON REMAINING	SUBSEQUENT MOULTS POSSIBLE	NOTES
737	NOV 80	YES	26.6	AUG 81	34.0	26.6→28.9→31.5→34.1	3	3 MONTHS	YES	CHELA REGEN.
774	JAN 81	NO	19.0	AUG 81	25.2	19.0→20.8→22.7→24.7	3	3 MONTHS	YES	
842	MAY 81	NO	15.5	SEP 81	22.7	15.5→17.0→18.6→20.3→22.2	4	2 MONTHS	YES	
863	JUN 81	YES	22.3	AUG 81	26.0	22.3→24.3→26.4	3	3 MONTHS	YES	

Crayfish number 737 was relatively large when first caught, and the fact that three, and possibly more moults occurred in 1981 may not have been indicative of the case for other large crayfish. It was regenerating a chela, and it has been demonstrated that this may result in a reduced intermoult period and increased moult frequency (e.g. Bennett, 1974; Brown, 1979 for review). This is presumably to compensate for the reduced moult increment at each moult due to the energetic demands of regenerating the lost limb.

The same method of analysing the moult frequency data of the females was employed, but using the appropriate model from Table 5.3. Animal number 720 was first caught in November 1980 at the end of the growing season. Its carapace length then measured 16.2 mm. In August 1981 it was subsequently recaptured, and measured 21.4 mm. Three moults would have been required, (16.2→18.0→19.9 →21.8). They will have occurred during the 1981 growing season. In November 1981, number 720 was again recaptured, and had moulted twice to 25.3 mm carapace length (21.4→23.4→25.5). Thus a total of five moults were observed to have occurred between November 1980 and November 1981.

Animal number 806 was first caught in February 1981. After three moults it was caught again in August 1981 having grown from 16.5 mm to 22.0 mm carapace length, (16.5→18.3→20.2→22.2). With three months of the growing season remaining, it is probable that subsequent moults may have occurred.

In conclusion, it appears from the above case studies and from examination of table 5.4, that for both males and females from the River Leen crayfish population, moult frequency is greater for smaller animals, and decreases with increasing size. Above the 22-24 mm size class females tend to moult less frequently than the males, and above 28 mm carapace length, none were observed to moult more than once a year. Females carrying eggs (two at size class 32-34 mm) moulted only once per year, and the reduction in the moult frequency observed for females around 22-24 mm carapace length and above, may have been due to an increasing proportion of the females becoming ovigerous. The minimum size observed for an ovigerous female was 23.1 mm (see 3.2). Those not carrying

eggs could moult more frequently, up to the 28 mm size class above which single moults were observed to occur irrespective of breeding state. For males, no such restrictions are imposed on multiple summer moults, although it will be seen that only two animals greater than 28 mm carapace length were observed to have moulted more than once.

For the Markfield population, no direct observations of moult frequency were possible, although it may be expected that since the growing season is shorter than for the River Leen, the moult frequency might also be less. The only method of predicting the annual moult frequency is thus the indirect method described above. This was applied to both populations and in the River Leen provides a good comparison with the observed moult frequencies for different size classes. For Markfield the results are more speculative. They are expressed in table 5.7 and those for the Leen are in Table 5.6, subsequent to the following section in which it is shown how these results are derived. A more detailed discussion of the predicted moult frequencies is presented in 5.1(iii).

POLYMODAL SIZE FREQUENCY ANALYSIS

METHODS

Histograms of size frequency have often been used to estimate the modal sizes of year classes, (e.g. Morriarty, 1971, 1972; Abrahamsson, 1972a; Huner and Romaire, 1978). However, it is often only possible to distinguish the first two, or sometimes three, year classes. Beyond this, variations in moult frequency and moult increment mean that overlap of the age classes occurs making them difficult to distinguish. A more rigorous approach is to

conduct a polymodal size-frequency analysis by plotting the percentage cumulative frequency against size (carapace length) on probability paper, (Cassie, 1954). Inflexion points then occur which represent the change from one modal size class to the next. This method has been used on studies of the Northumberland population of *A. pallipes*, (Brown and Bowler, 1978; Brown, 1979; Brewis and Bowler, 1982). It was concluded that the modal sizes achieved were a true representation of the year classes. Thus it is valid to state that mode one represents year class 0+, mode two represents year class 1+, and so on.

The problem of overlap of the age classes seen in the analysis of histograms also occurs with the method of polymodal size-frequency analysis. The problem arises from interpretation of the inflexion points. In this study, inflexion points were considered to have occurred where an obvious change in direction of the line occurred, or if it became almost vertical for a short distance. In this way the 0+, 1+ and 2+ year classes were easily described. It was sometimes also possible to distinguish the 3+ year class (4th mode) but this was often less obvious and therefore less credence should be attributed to these results.

Overlap between year classes is easily recognized using this method of analysis. Each mode, distinguished by the inflexion point, is separated out. The frequency of each size represented in that mode is then calculated and also plotted onto probability paper. Overlap becomes apparent when deviation of certain sizes occurs away from the line describing that mode. Thus it is possible to include the sizes which exhibit overlap into the calculations for the two modes implicated (see Cassie, 1954). Normally the

mean modal size is then read from the graph at the 50% level, and the standard deviation calculated. To make this quicker, and more simple and accurate, frequencies were converted to probits (Tables at the 0.001 level are available in Finney, 1952) and then regression against size was conducted using a desktop calculator. The mean modal size and standard deviation were computed.

Polymodal size-frequency analysis was conducted primarily on the overwintering populations, i.e. by combining the results for November to May inclusive. All animals caught during these months are in the dormant-overwintering phase, and so are all at the same moult stage (see 4.2). The sexes were treated separately for analysis.

During the summer growth period, animals caught in any one month may be at different stages of moulting. Thus analysis of size frequency data is complicated by the occurrence of increased overlap between size classes. Each mode represents the mean of some moulted and some, as yet, unmoulted animals. The information gained by analysis of the summer months, treated individually, is, however, of some interest. For the study of growth, it will give an indication of the average size increases observed for a year class as a whole, although it will not represent the growth of an individual. For examination of the population structure, it provides details of the percentage of the total population represented by a particular year class from one month to the next, the details of which have been presented in chapter 4 .

Analysis of the summer growth period was conducted in this way for the Leen population. It was not done for the Markfield population for several reasons. Both trapping and diving failed

to obtain a complete size range of animals, the majority of which were large. Hence more overlap was observed to occur between modes, and the inflexion points were often impossible to distinguish. Also, since the monthly collections were only made by trapping, the catches were too low for accurate analysis. Combined with this, the distorted size and sex distribution achieved by trapping methods, (see 2.1(ii)) only served to further exacerbate the problems outlined.

RESULTS

Figures 5.5a, and b, and Figure 5.6, show the histograms which represent the size distributions occurring for winter populations of the River Leen during 1980 and 1981, and Markfield Quarry during 1981, respectively. Three modes are easily distinguished for both sexes of the 1980 Leen population, and for the males of the 1981 population. The situation occurring with the 1981 Leen females is less clear, and that of the Markfield population also. For the Markfield males it appears that there may be three size classes, and two for the females. In an attempt to separate these modes more clearly the analysis described was employed, and the resulting graphs are shown in Figures 5.7 a-d for the Leen population, and Figures 5.8a, and b, for the Markfield population. The solid line represents the increasing percentage cumulative frequency, whilst the modes occurring between inflexion points (indicated by the arrows) are represented by the broken lines. Within each mode the frequency expressed by each size interval is calculated, and is shown by the open circles. An example of where overlap is occurring between modes is illustrated in Figure 5.7b. The last point in mode two deviates from the

line, so may also be included in the calculations for mode three. In general, very little overlap was observed, and for the River Leen populations the inflexion points are fairly obvious. This was not the case for the graphs of the Markfield population.

A comparison of the results of the River Leen population, expressed in Table 5.5, reveals that for the male sub-populations of the two years, no significant differences existed. This was the case for all year classes examined. Thus no differences in growth rate are observed to have occurred between 1980 and 1981 for the males. Comparing the sizes of the female year classes, however, significant differences result between both the 2+ ($P < 0.05 > 0.25$) and 3+ ($P < 0.025 > 0.01$) year classes. It is arguable as to whether this signifies a true difference in the growth rates between the two years. No difference was observed for the males, and also no obvious differences were observed in environmental conditions, particularly temperature (see 4.2) which is most likely to affect growth (see 5.1(iii)). It is thought that although the modes representing the year classes for the 1981 data are clear, consideration of the histogram in Figure 5.5b reveals that very few animals make up these modes in that year, and so the results obtained may be false due to the paucity of information. Thus it is felt that most credence should be attributed to the results of the 1980 analysis for the Leen females.

A comparison of the sexes reveals no significant differences at any size class for the 1980 population. Differences were observed between the 2+ and 3+ year classes for 1981, but this may be due to the paucity of information in 1981 for the female sub-

population, as argued above. Since no differences were observed to occur between the males of either year, a further comparison was made between the 3+ year classes, using data for the 1981 males and 1980 females (no 3+ year class was apparent in 1980 for the Leen males). No difference was observed. Thus it appears that over the four size classes separated out, which include mature animals, (see 3.2) no differences exist between the growth rates of males or females from the River Leen population.

The analyses which were conducted for catches of the Leen population made during the summer months are expressed in Chapter 4.2 where they relate to the population structure. The results relevant to the growth of *A. pallipes* are summarized in Figure 5.9a, and b. Growth is traced over the two year study period. Considerably more variation is observed to occur for the females than the males, but the results tend to support the view that, certainly for the 3+ year class, the 1980 winter result is the more correct. (A decrease in growth observed in the 1981 winter period, which is illustrated, would not occur).

By November, the average size of each age class has reached that of the winter population, thus indicating that moulting effectively ceases during October. The start of the growing period in May is indicated by the sudden increase in size observed between May and June. This is apparent in the 0+ and 1+ size classes for both males and females. That the larger size classes enjoy a shorter moulting season is illustrated in both sexes. The sudden increase in the average sizes of year classes 2+ and 3+ is not observed to occur until July. (Later moulting of mature animals was also reported in Chapter 3). From examination of

Fig. 5.9b for the females, it seems that the moulting period may also be shorter at the latter part of the season for large animals. The average size of the 2+ and 3+ year classes tends to reach the winter maximum by October, indicating that the majority of moults have been completed before the end of September. This position is not so clear for the males, for which separation of modes 3 and 4 (Yr classes 2+, 3+) was not possible in October 1980. That of year class 2+ in October 1981 is below the expected size for the 2+ winter population, indicating that moulting may not be complete. A further indication of the reduced moult frequency in the larger size classes is indicated in the smaller difference observed between the sizes of the 2+ and 3+ year classes than is exhibited by the 0+, 1+, and 2+ classes. This is apparent from Figs. 5.9a, and b.

Thus it will be seen that for the River Leen population, it was possible to separate out the modal size classes for the complete size range of crayfish occurring. It has been demonstrated that these modes clearly represent year classes, (Brown, 1979) and this is also supported by the study presented here. Evidence to support this view is obtained from comparison of the sizes of the separated modes (in Table 5.5), by assessing the predicted moult frequencies between modes (from the models in Table 5.3), and relating them to the observed moult frequencies (in Table 5.4). In this way it is possible to construct a predicted pattern of growth for *A. pallipes* in the River Leen as has been done in Table 5.6. The good correlation between the predicted and observed moult frequencies, suggests that not only are the modes accurate representations of year classes, but also that the models

in Table 5.3 used to predict growth are reasonably accurate.

For the Markfield quarry population, the size range available was not complete. Accordingly, mode one does not represent year class 0+, etc. The analysis of the Markfield data illustrated in Figs. 5.8a, and b, reveals that the inflexion points are less obvious than those observed for the Leen population, and so may not represent true year classes. If they do represent year classes, then it is not possible to state to which years they belong, solely on the analysis presented. Also it is not apparent whether the modes exhibited for the males are equivalent to those for the females, which precludes a comparison of growth between the sexes.

A similar predicted growth pattern to that for the Leen animals was constructed for the Markfield population, and it was attempted to deduce to which year classes the modes might belong. This is represented in Table 5.7. The postulated growth patterns given require detailed knowledge of moult increment analysis, moult frequency analysis, and size-frequency analysis. The size at which the Leen growth table begins is 5.36 mm, that observed for free juveniles in July. These animals had already moulted once from hatchlings, which were not observed in the field (hatchlings reared in the laboratory had a carapace length of about 4.5 mm). The next size given is calculated from the relevant Hiatt growth diagram, and the process is repeated until agreement is reached with the size obtained for the first year class by polymodal-size-frequency analysis. Subsequent year classes are treated similarly. Thus, the predicted moult frequency may be obtained and compared with that observed.

Above age class 3+, no size-frequency data is available, and so the growth is predicted by assuming only one moult per annum. This is based on the observations in Table 5.4 for the moult frequency of larger animals. A reduction in the maximum life span predicted would result if, as has been suggested for the larger males, the moult frequency was greater than one. The maximum life span is derived from observations of the largest animals caught, these being 49 mm (carapace length) for Leen males giving a predicted life span of 9 years, and 46 mm for Leen females, giving a life span of 10 years.

This method of predicting the growth of *A. pallipes* in the River Leen appears to provide a reasonably true picture of the real situation. However, Table 5.7 for the Markfield Quarry population is based more on speculation than observation. The reason for this is the limited size range mentioned previously, coupled with the lack of observation of the moult frequency. The "assumed moult frequency" given is based upon observations for the Leen population, but reduced to account for the shorter growing season at Markfield quarry (see 3.2). Furthermore, since no juveniles of the 0+ year class were ever caught, the starting size for the table was taken to be that of the Leen juveniles, since it is likely that the hatchlings of each population would have been approximately the same size.

5.1(iii) DISCUSSION

The growth pattern of decapod crustaceans such as *A. pallipes* appears at first sight to be relatively simple. However, to fully describe the events and changes which occur, several variables must be taken into consideration, and the overall picture resulting

then becomes quite complex. External factors such as temperature, photoperiod, food availability, and population density have all been shown to exert an influence on growth, (see below). These influences may act upon the moult increment occurring at ecdysis, or upon the moult frequency, or both. In addition, these latter variables are themselves not constant, even under constant environmental conditions, and changes are observed to occur with increasing size and age. The reproductive state of an animal, whether it has a full complement of appendages, or whether diseased, must also be considered, since these factors also may exert an effect on the growth rate.

Compared with laboratory studies, field studies of decapod growth, can obviously have no element of control over the environmental conditions. Hence, descriptions of the pattern of growth are based entirely upon analysis of moult increment, and moult frequency data, usually from marked, or tagged, released and recaptured animals. These analyses have generally employed one or more of the three methods previously outlined for moult increment data. Polymodal size frequency analysis has also been used on occasions. Thus, to take into account any environmental effects upon growth, comparisons of equivalent studies from different areas are necessary. It is the intention of this author to compare the Midlands populations of *A. pallipes* with growth data available for Northern and Southern populations in England (see below).

This study reported considerable variability of the absolute moult increment. Due to this, and to the relatively small sample size, comparisons of both the moult increment, and the growth factor against the pre-moult carapace length proved to be

statistically insignificant. Considerable variability of the moult increment has also been reported by many other authors working with decapod crustaceans (e.g. Hiatt, 1948; Edwards, 1965; Sather, 1966; Hopkins, 1967; Rumyanstev,1970; Flint, 1975; Pollock and Roscoe, 1977; Price and Payne, 1978; Brown, 1979; Rhodes, 1980). The overall trends reported by this author, however, were that no significant change in the moult increment occurred with increasing size (except for the Leen males, which showed an increase) and that the growth factor was observed to decrease with increasing size. Similar results are reported in the literature, which note a decreasing growth factor with increasing size (*A. pallipes*: Brown, 1979; Pratten, 1980; Rhodes, 1980; Brewis and Bowler, 1982. *Cancer pagurus* : Bennett, 1974. *Homarus vulgaris:* Simpson, 1961. *Nephrops norvegicus :* Farmer, 1973. *Paranephrops planifrons:* Hopkins, 1967. *Rhithropanopeus harrissii*: Turboyski, 1973). The picture for the absolute moult increment is not so clear. It has been reported to increase with increasing age/size (*Cancer pagurus*: Edwards, 1965. *Homarus vulgaris* : Simpson, 1963. *Nephrops norvegicus*: Farmer, 1973), remain constant with increasing size, (Lobster: Wilder, 1963. *Homarus vulgaris*: Hewett, 1974) or decrease with increasing size (*Astacus astacus:* Abrahamsson, 1971, 1972a, 1972b. *A. pallipes* (adults): Brown, 1979; Brewis and Bowler, 1982. *Jassus tristanii*: Pollock and Roscoe, 1977. *Pacifastacus leniusculus*: Abrahamsson, 1971).

The observation that the growth factor decreases appears to be common to all studies. Mauchline (1976) has argued that this decrease occurs logarithmically, indicating a curve. Similarly, Hopkins (1967) concluded that the growth factor showed a curvilinear

trend, decreasing with increasing carapace length. Templeman (1940), and Wilder (1963) showed that a curve best described the growth factor when larvae and large lobsters were plotted on the same graph. Brown (1979), working with *A. pallipes*, has argued that two linear regression lines fit the data the best, one describing the growth factor for juveniles, and the other, mature crayfish. However, Pratten (1980) has shown that for *A. pallipes* from the River Ouse, this data conforms very well to a log. linear transformation, again implying a curve.

In 5.1(i) it was stated that for the Hiatt growth diagram to be a straight line, the growth factor would have to either remain constant, or else change at a constant rate, i.e. conform to linear regression. Clearly in most cases it does not. Thus a curve is implicated when plotting the PrMCL against the PoMCL. In this study the decrease of the growth factor was in fact linear, but the result was not statistically significant. Hence, both linear and curvilinear (log.log.) regressions were attempted on the data of the Hiatt growth diagram. It was found that a curve best described the situation, as was predicted.

Linear regression analysis of the Hiatt growth diagram for Crustacea has been widely employed. Some describe the growth of males and females (either singly, or combined) as single regression lines (e.g. Wilder, 1963; Farmer, 1973; Flint, 1975; Mason, 1974). Others describe the relationship with two lines, which occur about an inflexion point, that has been described as the size around which sexual maturity occurs (e.g. Hiatt, 1948; Kurata, 1962; Brown and Bowler, 1978; Brown, 1979). In some cases this linear interpretation of the data may be the

correct one. Farmer (1973) produced a Hiatt growth diagram using large lobsters, but found that the juveniles fell exactly on this line, indicating that no change occurred in the growth factor with increasing size or age. In the majority of cases, however, linear analysis, although not incorrect, will not fully describe the true situation which is occurring, and so will not provide all the information which may be gained from curvilinear analysis. The fitting of two linear regression lines to the data to overcome this problem is not correct, and the inflexion point is an artefact arising from the shape of the curve (Mauchline, 1976).

This author did in fact fit two lines to the Leen data for comparison with that of Brown (1979) who described an inflexion point at 28 mm carapace length for both male and female *A. pallipes* in a Northumberland aqueduct. Sexual maturity was reported to occur at 22 mm carapace length for males and 25 mm carapace length for females, and the difference between these observations and that of the inflexion point was argued to be due to the fact that not all animals reach sexual maturity at the same time. For the River Leen population, the inflexion points, (deduced as the point at which the least value of the sums of squares for the residual errors occurred) were recorded at 27 mm and 25 mm for males and females respectively. Sexual maturity occurs at about the same sizes as for the Northumberland population (see 3.2). The fact that the inflexion point of the male data was greater than that for the females, and that the female result was at the size recorded for sexual maturity, tends to oppose the above argument, and also support the view that the inflexion point is an artefact of the curve.

The representation of the PoMCL against the PrMCL in a curvilinear fashion means that the growth coefficients described by Kurata (1962, see 5.1(i)) are no longer valid. His three types of growth rate, progressive geometric growth, retrogressive geometric growth, and arithmetic growth, effectively describe growth as increasing, decreasing, or constant, respectively. When the Hiatt growth diagram is treated as two lines about an inflexion point it is often found that juveniles possess progressive geometric growth, whilst adults are described by either arithmetic or retrogressive geometric growth (e.g. Kurata, 1962; Brown, 1979). This information, however, is all conveyed in the nature of the curve. It shows that growth increases initially, and then with increasing size it slowly decreases, or "decelerates".

The gradual and continual deceleration of growth which is observed to occur in *A. pallipes* may be due to the fact that more energy is required for other processes. For example, the females require to divert energy to the production of eggs, whilst the males grow increasingly large chelae as they get older, (see 5.2). The regeneration of new chelae has certainly been found to affect growth, altering both the absolute moult increment, and the timing or frequency of moulting (e.g. Hiatt, 1948; Kurata, 1962; Bennett, 1974; Brown, 1979).

No significant differences were observed between the growth rates of male and female crayfish from the River Leen. This was true when considering the absolute moult increment, the Hiatt growth diagrams, and the full range of modal sizes separated out for males and females. Pratten (1980) also reports this to be the case for *A. pallipes* in the River Ouse. For both populations,

however, differences may occur in mature crayfish in respect of moult frequency, which would result in the males of any one year growing more than the females. For the Markfield population, although no significant difference was observed between the sexes by comparison of the Hiatt growth diagrams, examination of the absolute moult increments revealed a significant difference. Similarly, Brown (1979) reported that significant differences occurred in the growth rates of adult male and female crayfish in Northumberland, although that for juveniles was similar. Comparison of the growth rates between the sexes in other decapod crustaceans reveals similarly that some populations do not have differential growth rates (e.g. *Cancer pagarus:* Edwards, 1965. *Nephrops norvegicus:* Farmer, 1973. *Pacifastacus leniusculus:* Flint, 1975. *Paranephrops planifrons:* Hopkins, 1967) whilst in others differences do exist between the sexes, (e.g. *Astacus astacus:* Abrahamsson, 1972a; Svärdson, 1949; Rumyanstev, 1970. *Jasus tristanii:* Pollock and Roscoe, 1977. *Homarus vulgaris:* Simpson, 1961).

Allen (1966) reviewed the growth of decapod Crustacea, and reported for some of the shorter lived species of the Natantia and Reptantia, that after egg laying has occurred the female has a higher moult frequency than the male so that it ends up being larger than a male of the same age. This is not the case with the longer lived decapods which carry their eggs for longer, and so the female only moults once a year. This describes the situation for *A. pallipes,* and as explained above, may result in reduced growth rate of females compared to males of the same age. Analysis of the observed moult frequency of animals from the River Leen revealed that several moults may occur each year

for both immature males and females. It was also shown that the moult season for immature animals is longer than that for adults, due to the fact that it starts earlier and finishes later. Hence a reduced moult frequency is observed with increasing size, and above 30 mm carapace length only one moult per annum was observed for both sexes, although it is possible that males may moult more frequently than this.

Moult frequency was also predicted (see tables 5.6, 5.7). Differences occurring between the observed and the predicted frequencies for Leen animals are slight. For a population of *A. pallipes* in the River Ouse, seven or eight moults were predicted for sub-yearling crayfish prior to the overwintering period (Pratten, 1980). In Northumberland this number is said to be six (Brown, 1979) and in a laboratory study of pooled Midlands populations the frequency was also observed to be six (Rhodes, 1980). Thus the prediction of seven moults for sub-yearling male crayfish is felt to be reasonably accurate. However, it is felt unlikely that six and five moults would occur in years 1+ and 2+, respectively, as predicted for the Leen males. The differences occurring here between the observed and predicted moult frequencies are probably due to the fact that the model describing the population is based mostly upon relatively large animals (see 5.1(ii)) and so does not extrapolate back as accurately as might be hoped. The predicted growth and moult frequencies recorded for the Leen female subpopulation fit extremely well to the observed situation.

For Markfield Quarry, no direct moult frequency data was available, and so the postulated growth pattern is based on supposition. The modes that were separated out by polymodal size-

frequency analysis do not appear to represent year classes in this case, unless a radically different moult frequency pattern is exhibited by the Markfield population. The predicted moult frequency between the modes was greater than would be expected for large crayfish, being three moults (from 31.4 to 36.24 mm) for the males, and two moults (from 28.73 to 32.39 mm) for the females. An alternative explanation could be that the modes do in fact represent year classes, but that the Hiatt growth diagram used to predict the moult frequency was incorrect. This could have occurred since the moult increment data used in the construction of the diagram was based upon animals which had been kept in holding tanks at the University. In this situation the moult increment may have been substantially less than it would have been in the wild. Reductions in the growth rates of laboratory held animals have been reported by several authors, (e.g. Hiatt, 1948; Simpson, 1963; Kurata, 1962; Hewett, 1974; Flint, 1975). This may also explain the fact that although no significant difference was observed between the growth rates of the Leen and Markfield populations when considering the Hiatt growth diagrams, a significant difference was observed between the mean absolute moult increments.

The possibility of errors occurring in the proposed growth pattern of the Markfield Quarry population, must be remembered when comparisons are drawn (below) with other populations of *A. pallipes*. These errors could form an alternative to the arguments presented in explaining any differences observed. The postulated growth pattern for crayfish from the River Leen, however, is felt to be an accurate representation of the real situation.

Comparison of the growth rates between *A. pallipes* and other crayfish species has been reviewed by Brown (1979). Since this study now provides the third comprehensive analysis of the growth data relating to *A. pallipes*, comparison is drawn between the data made available here, and that in the literature. The results are summarized in Table 5.8. Those relating to the Irish population (Morriarty, 1971) are based chiefly upon speculation and no detailed growth study was conducted. Since no 0+ juveniles were caught for this population, and the 13 mm, 16 mm, and 25 mm size classes relate to observations not included in the catches, which all consisted of crayfish 25 mm carapace length and above, the modes identified probably do not represent the year classes shown. Comparison with the other data available would indicate that crayfish of the sizes identified (35-42 mm) should in fact be placed in later year classes. All other data in the table is based upon more rigorous examination of the situation occurring, and so the results are directly comparable.

When considering the growth of Crustacea, temperature has been observed to be of primary importance. It affects both the timing of moulting, and the moult frequency, (Svärdson, 1949; Kurata, 1962; Momot, 1964; Allen, 1966; Abrahamsson, 1971, 1972a; Turboyski, 1973; Huner and Romaire, 1978; Mason, 1978; Richards and Wickins, 1979; Rhodes, 1980). It has also been argued that it will affect the absolute moult increment, since lower temperatures will result in a reduction of feeding activity (Abrahamsson, 1972a).

For each English crayfish population reported, the temperature range during the growing season starts at about the same value,

10°C or 11°C. The maximum temperatures vary considerably, but these occur in the middle of the growing season, and it is the lower temperature value, which will occur at either end of this period, which will delimit its length. It will be seen that the Southerly populations exhibit the longest periods of growth, extending from mid-May until late October. They also have the greatest range of temperatures. The River Leen population shares a similar temperature range and growth season, and accordingly growth rates and moult frequency are not dissimilar to those reported for the population in the River Ouse. The size of the hatchlings at the end of the first summer is about 10 mm in the Leen compared to 13 mm for the Southerly populations. This may be due to the fact that the eggs hatch later in the Midlands population. Hence it is likely that they may undergo one less moult than those in the Rivers Ouse or Darent.

The second Midlands population studied, that of Markfield Quarry, has a shorter growing season than that of the Leen, or the Southerly populations, but longer than that in Northumberland. The temperature range is also lower. Differences exist between the two Midlands populations in this respect, not because of climatic variations, but due to the fact that the large body of water at Markfield Quarry takes considerably longer to warm up than the shallow water of the River Leen. Cooling of the water would also be expected to take longer, which would perhaps result in similar growing periods for the two populations. However, the maximum temperature in the Quarry during the study period did not exceed 17°C (however, see Table 2.1), compared with 20°C in the Leen. Due to the shorter growth period, it will be seen

that the Markfield Quarry population forms an intermediate between those in the South, including the River Leen, and that in the North of England. Moult frequency appears similar to the Northumberland population, but again, the earlier hatching of the juveniles, at the beginning of July for the Markfield population, compared with late August in Northumberland, results in a larger size at the end of the first growth period (9.3 mm at Markfield, compared to 8.5 mm in Northumberland).

Photoperiod has also been implicated as a factor which may affect the growth of decapod crustaceans. Hormonal control of the moult cycle has been demonstrated (Willig and Keller, 1973) and it is suggested that the photoperiod acts by regulating synthesis or release of a moult inhibiting hormone, which in turn will alter the response of the tissues to a moult hormone (Aiken, 1969). Ecdysis occurs when the balance moves sufficiently in favour of the moult-hormone. Stephens (1955) has clearly shown that in *Cambarus* moulting may be induced by altering the photoperiod at constant temperatures. That temperature is not the only factor involved is also demonstrated by Turboyski (1973), who found that crabs maintained at warm temperatures over the winter did not moult. This is not the case with *A. pallipes*, for which continual year-round moulting has been demonstrated under laboratory conditions (Brown, 1979; Rhodes, 1980). Nevertheless it may be that photoperiod is affecting the timing of the initial moult occurring in natural conditions. The first observation of moulting in the River Darent was early May. It was also May in the River Ouse, late May in the River Leen, but late June for the Northumberland population. This would show the opposite of the expected trend if photoperiod

was the sole regulator of moulting, since the day length would increase first in the North of England. In addition, it will be seen that Markfield population does not start moulting until about three weeks after the Leen population. This would imply that temperature is of greater importance than any effects of photoperiod, although it is possible that these two factors may combine to induce moulting when together they produce the optimum conditions for a moult.

Food availability has been shown to affect the moult increment at Ecdysis (Svärdson, 1949; Abrahamsson, 1972a; Clark and Avault, 1974; Hewett, 1974; Huner, *et.al.*, 1974; Newman and Pollock, 1974). It may also affect the interval between moults (Kurata, 1962). Additionally, it has been argued that the differential growth rates sometimes observed between the sexes may be due to the reduced feeding activity of ovigerous females (Abrahamsson, 1972a). For the Northumberland population of *A. pallipes* it has been shown that the decreased growth rate of the females is not confined to ovigerous animals. Thus it may be possible to extend the argument and say that the reduced growth of females may be due to the dominance of males over females, affecting food searching (see Bovbjerg, 1953).

It is probable that all the sites recorded had a similar food availability, although in Northumberland very little allochthonous organic detrital matter was available. Hence, food may have been at more of a premium than in the other populations, which in terms of the above argument could explain the differential growth rates observed between the sexes. No such differences existed for animals from the Rivers Leen or Ouse, except due

to differences in moult frequency, but were observed for the Markfield Quarry population.

Another factor reported to affect the growth of Crustacea is population density (Svärdson, 1949; Abrahamsson, 1966, 1972a; Hopkins, 1967; Clark and Avault, 1974; Huner, *et. al.*, 1974; Huner and Romaire, 1978). Stunted populations result from overcrowding, and this is possibly linked to food availability. Thus in crowded populations one might also expect to find more differences in the growth rates of the sexes. Certainly the growth rate of the Leen population is greater than that of either of the Markfield or Northumberland populations, and it is relatively sparsely populated in comparison with them. Differences between the growth of the sexes are also not exhibited by crayfish in the Leen, unlike those from Markfield or Northumberland. Another argument, however, may also apply. Given an adequate food supply, the absolute population density may not be the limiting factor. Instead it may be the availability of suitable hides. Richards and Wickens (1979), have shown that the provision of shelter for growing lobsters increases their moult increment at ecdysis.

Cannibalism at moulting has been reported commonly, (see Rhodes, 1980 for review; Goddard, 1982). Thus, adequate shelter becomes essential. Additionally, the first moult of the year appears to occur synchronously for a large number of animals. This was observed to be the case for the Midlands populations, and also those in the Ouse and Northumberland. No evidence was found that subsequent moults occurred synchronously, except by Pratten (1980). Should this be the case, however, it would certainly be of considerable survival value by reducing the effects of

cannibalism at the moult, especially where shelter was scarse.

Comparing the sizes for hatchlings, and at sexual maturity, it will be seen that all the populations are very similar. Differences occur in the time taken to reach sexual maturity due to the differential growth rates between the populations. Each consisted of the same species of crayfish *Austropotamobius pallipes* and it is felt that although genetic differences may occur, it is the environmental factors discussed which have exerted the most influence, and have resulted in the differences observed. The maximum life spans recorded are similar, (between 9 and 12 years) although some variation occurs in the maximum size attained. The smallest was at Markfield Quarry, which also has the densest population, so this may be a density effect.

It was stated at the beginning of this chapter that if the British crayfish were to form a viable alternative to foreign crayfish species for the luxury food market, then a comparison of the time scales involved in reaching a marketable size would be necessary. What constitutes a 'marketable size' varies to a certain extent. For *Astacus astacus* the minimum legal size is 90 mm total length in France, (Laurent, 1972) and Sweden, (Svärdson, 1949). This is to protect the species. No such protection is deemed necessary for *Cambarus* sp. in France, so no legal size limits have been set (Laurent, 1972). For *Pacifastacus leniusculus* marketable size is also 90-100 mm (Karlsson, 1977), and Avault *et. al.*(1974) report that the preferred size for *Procambarus clarkii* is 100 mm although crayfish of 75 mm may also be sold. In general then, it appears that 90-100 mm total length constitutes a marketable size.

Since the decline of the gastronomically preferred *Astacus astacus* in Europe, several crayfish species have been sold. *Astacus leptodactylus* has been imported from Turkey, and *Orconectes limosus* has also been eaten (Laurent, 1972). However, the preferred species appears to be the signal crayfish, *P. leniusculus*, native to Western Canada and the North-western United States. It has been reported to have the same "excellent taste" as *Astacus astacus* (Karlsson, 1977). It is a faster growing species than the European crayfish, and may reach sexual maturity and marketable size by the end of the second growing season, (Abrahamsson, 1971; Brinck, 1974; Karlsson, 1977; Goddard, 1982). It takes *Astacus astacus* at least three growing seasons to reach this size, whilst *Procambarus* sp. may take only two or three months to reach adult size (Payne, 1978). This is dependent upon the time of hatching, and if this occurs in December growth may take longer, but they will have reached marketable size by May of the following year, i.e. five or six months (Avault *et.al*, 1974).

The carapace length is almost exactly half the total length of *A. pallipes* (see 5.2). Thus marketable size relates to crayfish of 45-50 mm carapace length. To reach this size would take eight years in the River Leen and Southern populations, and nine years for the Northumberland population. For the Markfield Quarry population, marketable size is approximately equivalent to the maximum size recorded, and so it would take eleven years for individuals of this population to become saleable. Possibly cropping of this population, thus reducing its density may result in an increased maximum size and growth rate, but even then the timescale would only be reduced slightly. Thus it may be seen that from

a commercial point of view, *A. pallipes* does not compare favourably with the growth rates of other crayfish species. In addition, it will be seen that only a minor proportion of the population is represented by size classes greater than the 3+ age group (e.g. 1.1+7.4% in the River Leen; see table 5.5), so even fewer would be represented by the 8+ (and above) age groups. A catch where 25% of the individuals were greater than 90 mm total length was achieved by Morriarty (1971) and so this could perhaps have proved to be of commercial value. In general, however, it is not felt that the cropping of natural populations of *A. pallipes* would prove commercially viable unless extremely high population densities existed. This would then have the drawback of resulting in stunted populations. Certainly the culture of *A. pallipes* must be discounted in favour of other faster growing species, although it has been suggested that by culturing juvenile *A. pallipes*, seeding of natural populations could then be carried out in order to maintain suitable stocks of larger animals (Goddard and Holdich, 1979; Rhodes and Holdich, 1979).

TABLE 5.1 A COMPARISON OF LINEAR AND LOG. LINEAR REGRESSION ANALYSIS FOR ABSOLUTE MOULT INCREMENT (Y) AGAINST PREMOULT CARAPACE LENGTH (x) AND ANALYSIS OF THE SLOPE OF THE BEST FIT LINE FOR SIGNIFICANT DEVIATION FROM '0'

POPULATION	SEX	EQUATION OF LINE	S.E.b.	r	F	df	P	BEST FIT	b DEVIATES FROM 0?
LEEN	MALES	Y = 0.0491x + 0.9956	0.0291	0.3138	2.841	26	> 0.1 < 0.25		t = 2.1739
		LOG.Y = 0.0150x - 0.0592	0.0069	0.3941	4.7816	26	> 0.01 < 0.05	YES	P<0.05>0.025
LEEN	FEMALES	Y = 0.0128x + 1.8148	0.0186	0.1211	0.4764	32	> 0.25		t = 2.1768
		LOG.Y = 0.01502x - 0.0591	0.0069	0.3941	0.4764	32	> 0.25	YES	P<0.05>0.025
MARKFIELD	MALES	Y = -0.0308x + 2.737	0.0553	0.1235	0.3099	20	> 0.25		t = 0.6070
		LOG.Y = -0.0102x + 0.5360	0.0168	0.1349	0.3710	20	> 0.25	YES	P>0.50
MARKFIELD	FEMALES	Y = -0.0541x + 2.9780	0.0534	0.2615	1.027	14	> 0.25	YES	t = 1.0131
		LOG.Y = -0.0134x + 0.4745	0.0237	0.1449	0.322	14	> 0.25		P<0.40>0.20

TABLE 5.2 A COMPARISON OF LINEAR AND LOG. LINEAR REGRESSION ANALYSES FOR GROWTH FACTOR (Y) AGAINST PREMOULT CARAPACE LENGTH (x) AND ANALYSIS OF THE SLOPE OF THE BEST FIT LINE FOR SIGNIFICANT DEVIATION FROM '0'

POPULATION	SEX	EQUATION OF LINE	S.E.b.	r	F	df	P	BEST FIT	b DEVIATES FROM 0?
LEEN	MALES	Y = -0.1085 x + 11.7110	0.1214	0.1726	0.7984	26	> 0.25	YES	t = 0.8937
		LOG.Y = -0.0041x + 1.0310	0.0064	0.1241	0.4070	26	> 0.25		P<0.40>0.20
LEEN	FEMALES	Y = -0.2631x + 15.4741	0.0736	0.5341	12.7709	32	< 0.01	YES	t = 3.5747
		LOG.Y = -0.01421x + 1.2762	0.0041	0.5189	11.7928	32	< 0.01		P<0.005>0.001
MARKFIELD	MALES	Y = -0.2530x + 13.6332	0.1615	0.3306	2.4545	20	<0.25 > 0.1	YES	t = 1.5666
		LOG.Y = -0.0235x + 1.4568	0.0168	0.2985	1.9560	20	<0.25> 0.1		P<0.20>0.10
MARKFIELD	FEMALES	Y = -0.2934x + 13.3406	0.1708	0.4172	2.950	14	<0.25> 0.1	YES	t = 1.7178
		LOG.Y = -0.0268x + 1.3973	0.0237	0.2888	1.273	14	< 0.25		P<0.20>0.10

TABLE 5.3 A COMPARISON OF LINEAR AND LOG. LINEAR REGRESSION ANALYSES USED TO DESCRIBE THE HIATT GROWTH DIAGRAM, i.e. THE RELATIONSHIP BETWEEN THE POSTMOULT CARAPACE LENGTH (Y) AND THE PREMOULT CARAPACE LENGTH (x)

POPULATION	SEX	EQUATION OF LINE	S.E.b.	r	F	df	P	BEST FIT
LEEN	MALES	Y = 1.0491x + 0.9956	0.0291	0.9901	1297.3	26	< 0.01	
		LOG.Y = 0.9869LOGx + 0.0553	0.0253	0.9916	1525.2	26	< 0.01	YES
LEEN	FEMALES	Y = 1.0128x + 1.8148	0.0186	0.9946	2962.9	32	< 0.01	
		LOG.Y = 0.9483LOGx + 0.1083	0.0167	0.9951	3221.9	32	< 0.01	YES
MARKFIELD	MALES	Y = 0.9692x + 2.7372	0.0553	0.9689	307.1	20	< 0.01	
		LOG.Y = 0.9210LOGx + 0.1421	0.0506	0.9711	330.8	20	< 0.01	YES
MARKFIELD	FEMALES	Y = 0.9456x + 2.9780	0.0534	0.9784	313.5	14	< 0.01	YES
		LOG.Y = 0.9072LOGx + 0.1563	0.0532	0.9768	290.9	14	< 0.01	

TABLE 5.4 A TABLE SHOWING THE NUMBER OF CRAYFISH FROM THE LEEN POPULATION WHICH HAD MOULTED A CERTAIN NUMBER OF TIMES DURING THE GROWING SEASON, BASED ON MARKED AND RECAPTURED ANIMALS

PREMOULT CARAPACE LENGTH (mm)	NUMBERS IN THE COLUMNS REFER TO THE NUMBER OF CRAYFISH MOULTING n TIMES								
	MALES				FEMALES				
	NO. OF MOULTS OBSERVED				NO. OF MOULTS OBSERVED				
	1	2	3	4	1	2	3	4	5
10 - 12		1			1		2		
12 - 14			1			1			
14 - 16	1	1	2	1	2	1	1		
16 - 18	1	3			2		5		1
18 - 20		3	2		1	4	1		
20 - 22		5	2			3	1		
22 - 24		3	2			3			
24 - 26	2	2	1			1			
26 - 28		3	1		1	4			
28 - 30	1	2			1				
30 - 32					5				
32 - 34	1				5				
34 - 36					3				
36 - 38	1								

TABLE 5.5 POLYMODAL SIZE FREQUENCY ANALYSIS OF WINTER POPULATIONS OF *A. PALLIPES* FROM THE RIVER LEEN AND MARKFIELD QUARRY

POPULATION	SEX	WINTER	YEAR CLASS	MEAN C.L.	S.D.	% POPn. IN YEAR CLASS	N	TOTAL	COMPARISON OF DATA, P = YR:YR	♂:♀
LEEN	MALE	1979/80	0+ 1+ 2+ 3+	9.36 17.90 26.22 -	1.29 2.45 2.45 -	21.86 47.92 26.04 4.17	21 46 25 4	96	> 0.5 > 0.4 > 0.5	> 0.2 > 0.05 > 0.10
LEEN	FEMALE	1979/80	0+ 1+ 2+ 3+ 4+	8.98 17.05 25.07 31.51 -	1.00 2.16 2.45 1.29 -	22.34 39.36 27.66 7.45 3.19	21 37 26 7 3	94	> 0.2 > 0.2 < 0.05 < 0.025	 > 0.025 > 0.01
LEEN	MALE	1980/81	0+ 1+ 2+ 3+ 4+	9.36 17.11 26.29 32.01 -	1.00 1.87 2.82 0.96 -	13.95 50.00 26.74 8.14 1.12	12 43 23 7 1	86		> 0.5 > 0.2 < 0.025 >0.01 < 0.025 >0.01
LEEN	FEMALE	1980/81	0+ 1+ 2+ 3+ 4+	9.35 16.38 23.25 28.88 -	1.29 1.87 1.92 2.22 -	16.07 44.64 14.29 17.86 7.14	9 25 8 10 4	56		
MARKFIELD	MALE	1980/81	- ? ? -	~ 27 31.40 36.24 -	- 1.87 1.29 -	8.51 48.94 38.30 4.26	4 23 18 2	47		
MARKFIELD	FEMALE	1980/81	? ? ?	28.73 32.39 -	1.00 1.29 -	17.39 52.17 30.43	4 12 7	23		

TABLE 5.6 THE POSTULATED GROWTH PATTERN OF *A. PALLIPES* FROM THE RIVER LEEN, RELATED TO AGE. PREDICTIONS OF GROWTH, AGE CLASSES AND MOULT FREQUENCY ARE BASED UPON MI ANALYSIS, POLYMODAL SIZE FREQUENCY ANALYSIS, AND COMPARISONS WITH OBSERVED MOULT FREQUENCIES

MALES				YEAR CLASSES	FEMALES			
OVERWINTER C.L. (mm)	PREDICTED GROWTH	PREDICTED MOULT FREQUENCY	OBSERVED MOULT FREQUENCY		OVERWINTER C.L. (mm)	PREDICTED GROWTH	PREDICTED MOULT FREQUENCY	OBSERVED MOULT FREQUENCY
HATCHLINGS	~4.50				HATCHLINGS	~4.50		
1980: 9.36 ±1.00 1981: 9.36 ±1.29	5.36 5.96 6.61 7.32 8.10 8.95 9.88	7	-	0+	1980: 8.98 ±1.0 1981: 9.35 ±1.29	5.36 6.31 7.35 8.51 9.78	5	-
1980: 17.90 ±2.45 1981: 17.11 ±1.87	10.89 11.99 13.18 14.47 15.87 17.39	6	4+	1+	1980: 17.05 ±2.16 1981: 16.38 ±1.87	11.16 12.63 14.22 15.91 17.69	5	5
1980: 26.22 ±2.45 1981: 26.29 ±2.82	19.02 20.79 22.69 24.74 26.94	5	3+	2+	1980: 25.07 ±2.45 1981: 23.25 ±1.92	19.57 21.54 23.58 25.69	3(FOR 1981) OR 4(FOR 1980)	3+
1981: 32.01 ±0.96	29.31 31.85	2	2	3+	1980: 31.51 ±1.29 1981: 28.88 ±2.22	27.88 30.12	2	2
- - - - - -	34.57 37.49 40.61 43.94 47.49 51.28	1 1 1 1 1 1	1 1 - - - -	4+ 5+ 6+ 7+ 8+ 9+ 10+	- - - - - - -	32.41 34.75 37.11 39.51 41.92 44.35 46.78	1 1 1 1 1 1 1	1 1 - - - - -

NOTES: (1) The size of hatchlings was not determined for animals from the field population. For laboratory reared animals this size was about 4.5 mm, and crayfish caught in July measuring 5.36 mm C.L. were presumed to have moulted once.

(2) The maximum sizes of crayfish caught were 48 mm for males, and 46 mm for females.

TABLE 5.7 THE POSTULATED PATTERN OF GROWTH FOR *A. PALLIPES* FROM MARKFIELD QUARRY

MALES				YEAR CLASSES	FEMALES			
OVERWINTER C.L.	PREDICTED GROWTH	PREDICTED MOULT FREQUENCY	ASSUMED MOULT FREQUENCY*		OVERWINTER C.L.	PREDICTED GROWTH	PREDICTED MOULT FREQUENCY	ASSUMED MOULT FREQUENCY*
-	5.36 6.51 7.79 9.19	4	-	0+		5.36 6.57 7.91 9.36	4	-
-	10.69 12.30 13.99 15.76	4	3+	1+		10.90 12.51 14.19 15.89	4	4
-	17.58 19.44 21.33	3	2+	2+		17.62 19.35 21.07	3	2+
~ 27	23.24 25.14 27.03	3	2+	3+		22.76 24.4 26.00	3	2
31.4 ±1.87	28.89 30.72 32.51	3	2	4+	28.7 ±1.0	27.54 29.01	2	1
36.24 ±1.29	34.25 35.94	2	1	5+	32.39 ±1.29	31.75 31.75	2	1
- - - - - - -	37.57 39.13 40.62 42.05 43.41 44.70 -	1 1 1 1 1 1 -	1 - - - - - -	6+ 7+ 8+ 9+ 10+ 11+ 12+	- - - - - - -	33.02 34.21 35.33 36.37 37.34 38.25 39.09	1 1 1 1 1 1 1	- - - - - - -

NOTES: Size frequency data is only available for animals >27 mm C.L. (♂) and >28mm C.L. (♀). Thus the beginning of this table is entirely speculative. So too, is the assumed moult frequency, which has been based on table 4.3 for the moult frequency of different size classes of crayfish from the River Leen, but reduced by one moult in each case to allow for the shorter growth period at Markfield Quarry (see 3.1). It was also assumed that juveniles would be approximately the same size at the start of the growth period as those from the River Leen (i.e. 5.36 mm).

TABLE 5.8 A COMPARISON OF SOME FACTORS INCLUDING GROWTH, OF DIFFERENT POPULATIONS OF *A. PALLIPES* IN THE BRITISH ISLES

LOCATION	HABITAT	FOOD AVAILABILITY	POPn. DENSITY 'n' m^{-2}	LENGTH OF GROWING SEASON	TEMP. RANGE DURING GROWING SEASON	EGGS LAID	JUVENILES REARED	SIZE OF HATCHLINGS	OVERWINTER SIZES FOR YEAR CLASSES (PARENTHESES INDICATE NUMBER OF MOULTS P.A.) 0+ 1+ 2+ 3+ 4+ 5+ 6+ 7+ 8+ 9+ 10+ 11+ 12+ 13+	MAX LIFE SPAN (- MAX SIZE) PREDICTED	MIN SIZE AT SEXUAL MATURITY	REFERENCES
RIVER DARENT, KENT (SOUTH)	RIVER, 60cm DEEP. SUBSTRATE MUD, STONES AND ROCKS.	ELODEA sp. PRESUMABLY ALSO DETRITUS & INVERTS.	-	MID MAY TO LATE OCTOBER	11 - 21°C	EARLY NOV.	EARLY JUNE	5-6mm LATE JUNE	1ST ♂ 12-13mm 2ND ♀		♂~22mm ♀23-27mm	(1)
RIVER OUSE (SOUTH)	RIVER, 25-100cm DEEP	ENTEROMORPHA, CLADOPHORA PRESUMABLY ALSO DETRITUS & INVERTS.	-	MAY TO OCTOBER	10 - 24°C	-	EARLY JUNE	4.5mm	♂ 12.7 20.0 24.3 29.0 33.8 36.3 38.8 (8) (5) (3) (2) (2) (1) (1) ♀ 12.7 20.0 24.3 29.0 31.4 33.8 36.3 (8) (5) (3) (2) (1) (1) (1)	(MAX SIZE 56mm) 47mm	♂ 25mm ♀ 25mm	(2)
RIVER LEEN (MIDLANDS)	RIVER 30cm DEEP. GRAVEL & LARGE STONES AND ROCKS.	CLADOPHORA DETRITUS, INVERTEBRATE SPECIES	2.2-3.6m^2	LATE MAY TO LATE OCTOBER	10 - 20°C	LATE OCTOBER	LATE JUNE/ EARLY JULY	4.5mm	♂ 9.9 17.4 26.9 31.9 34.6 37.4 40.6 43.9 47.5 51.3 (7) (4+) (3+) (2) (1) (1) (1) (1) (1) (1) ♀ 9.8 17.7 25.7 30.1 32.4 34.8 37.1 39.5 41.9 44.4 46.8 (5) (5) (3+) (2) (1) (1) (1) (1) (1) (1) (1)	9 YRS (49mm) 10 YRS (46mm)	♂~25mm ♀23-25mm	(3)
MARKFIELD QUARRY (MIDLANDS)	QUARRY 8m DEEP. LARGE ROCKS & MUD BOTTOM.	CLADOPHORA DETRITUS INVERTEBRATE SPECIES	6-8m^2	MID JUNE TO LATE SEPTEMBER	11 - 17°C	-NOVEMBER	LATE JUNE/ EARLY JULY	-	♂ 9.2 15.8 21.3 27.0 32.5 35.9 37.6 39.1 40.6 42.1 43.4 44.7 (4) (3+) (2+) (2+) (1) (1) (1) (1) (1) (1) (1) (1) ♀ 9.4 15.9 21.1 26.0 29.0 31.8 33.0 34.2 35.3 36.4 37.3 38.3 39.1 (4) (4) (2+) (2) (2) (2) (1) (1) (1) (1) (1) (1) (1)	11 YRS (44mm) 12 YRS		(3)
NORTHUMBERLAND AQUEDUCT (NORTH)	AQUEDUCT 1.5m DEEP LARGE STONES & SANDSTONE BLOCKS.	FONTINALIS INVERTEBRATE SPECIES, LITTLE DETRITUS	7m^2	LATE JUNE TO EARLY SEPTEMBER	10 - 17°C	MID-LATE NOVEMBER	LATE AUGUST	4.6mm	♂ 8.4 13.4 18.8 26.0 31.9 34.9 37.9 40.8 43.5 46.1 48.4 50.3 (5) (4) (3) (3) (2) (1) (1) (1) (1) (1) (1) (1) ♀ 8.5 12.8 19.0 25.2 30.3 32.8 31.3 37.6 39.7 41.6 43.3 44.8 46.1 (6) (4) (4) (3) (2) (1) (1) (1) (1) (1) (1) (1) (1)	11 YRS (50.5mm) 12 YRS (45.6mm)	♂~22mm ♀ 26mm	(4)
WHITE LAKE (IRELAND)	LAKE, 15m DEEP. WHITE CALCAREOUS MUD		(1,200ha^{-1}) ≡ 0.12m^{-2}	LATE JULY TO SEPTEMBER	15 - 20°C	NOVEMBER	MID JUNE	-	13m [16 25 39 42] *SEE TEXT [16 25 35 37] *		♀~28mm	(5)

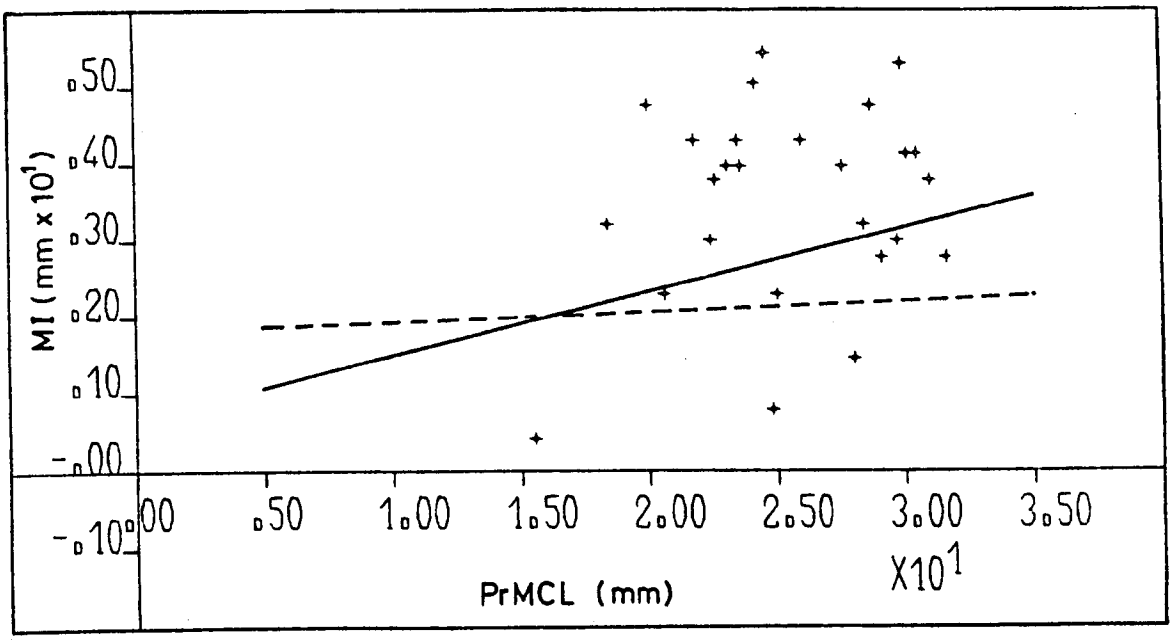
REFERENCES: (1) THOMAS AND INGLE (1971). (2) PRATTEN (1980). (3) AUTHOR. (4) BROWN (1979). (5) MORRIARTY (1972)

FIGS. 5.1 a-d

These computer drawn figures show moult increment (MI) plotted against the premoult carapace length (PrMCL) for the River Leen (males, a, females, b,) and Markfield Quarry (males, c, females, d) populations. Note the great scatter of points and large variability of MI at all carapace lengths. the lines fitted are the best fit linear regression data (see Table 5.1) although a log. linear relationship most closely resembles the actual situation, and only the analysis of the River Leen males proved to be statistically significant. The broken line in each Figure is that of the opposite sex for that population, transposed for means of comparison.

FIG. 5.1a-b RIVER LEEN POPULATION, MALES (a), FEMALES (b)

(a)



(b)

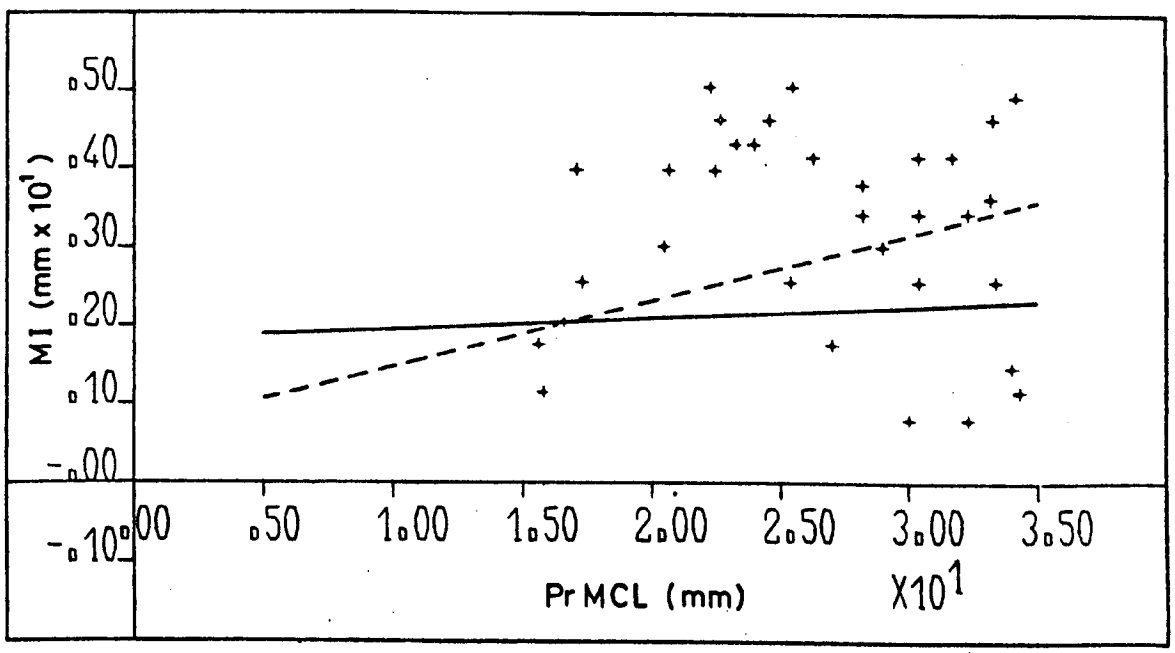
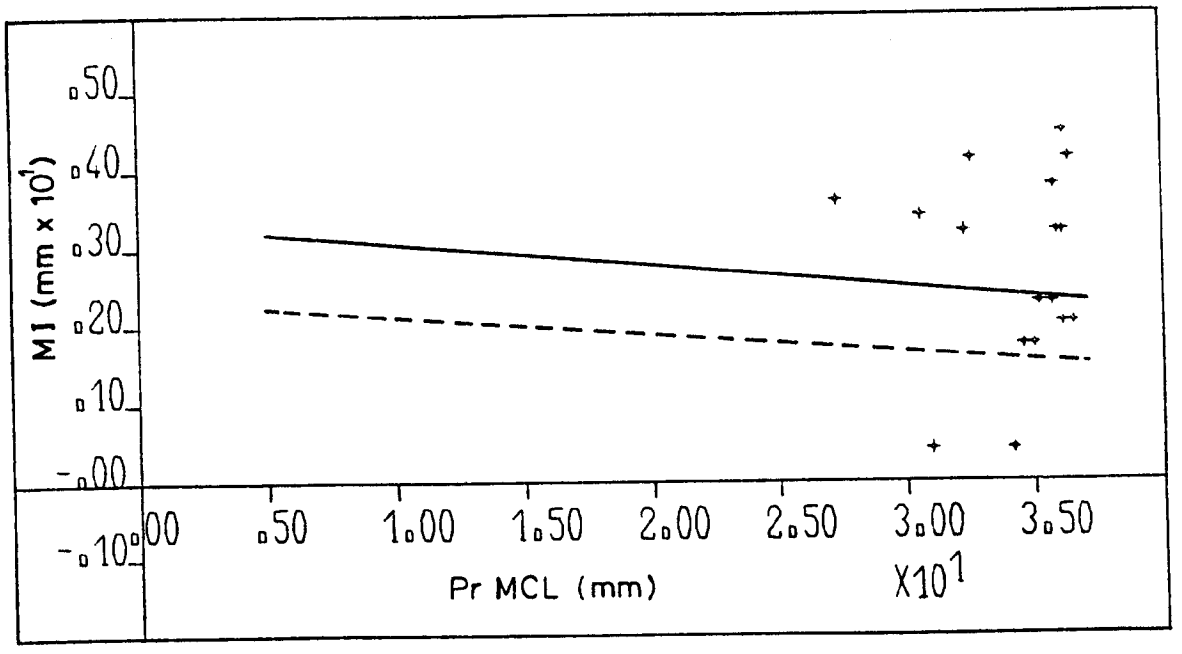


FIG. 5.1c-d, MARKFIELD QUARRY POPULATION, MALES (c), FEMALES (d)

(c)



(d)

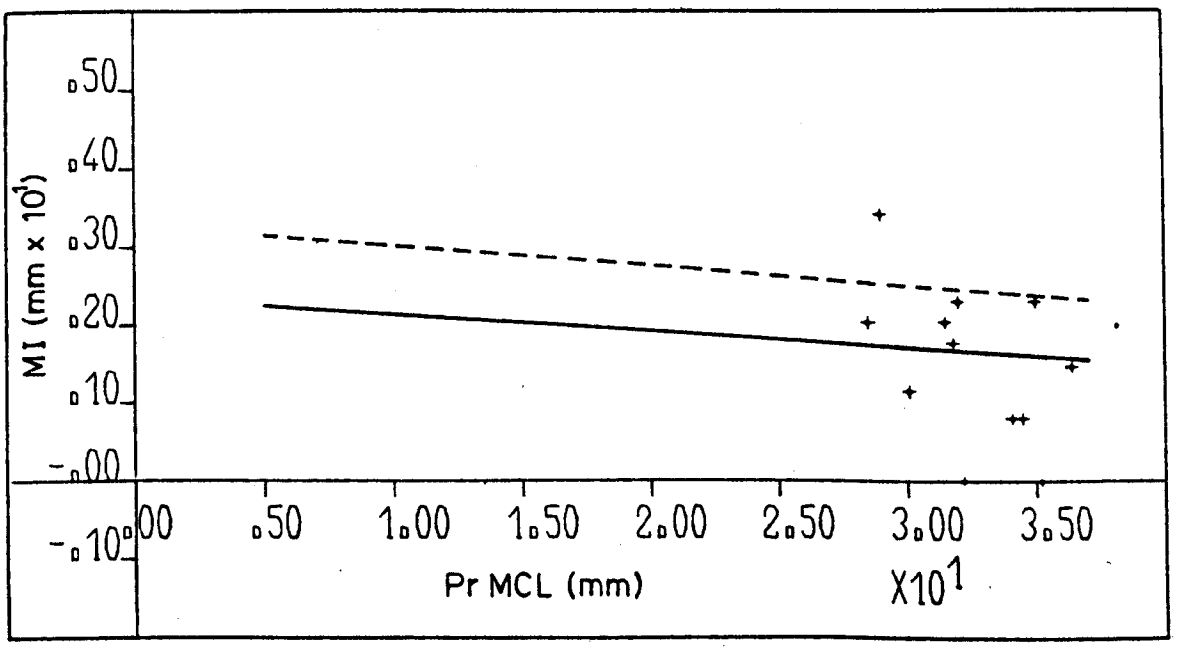
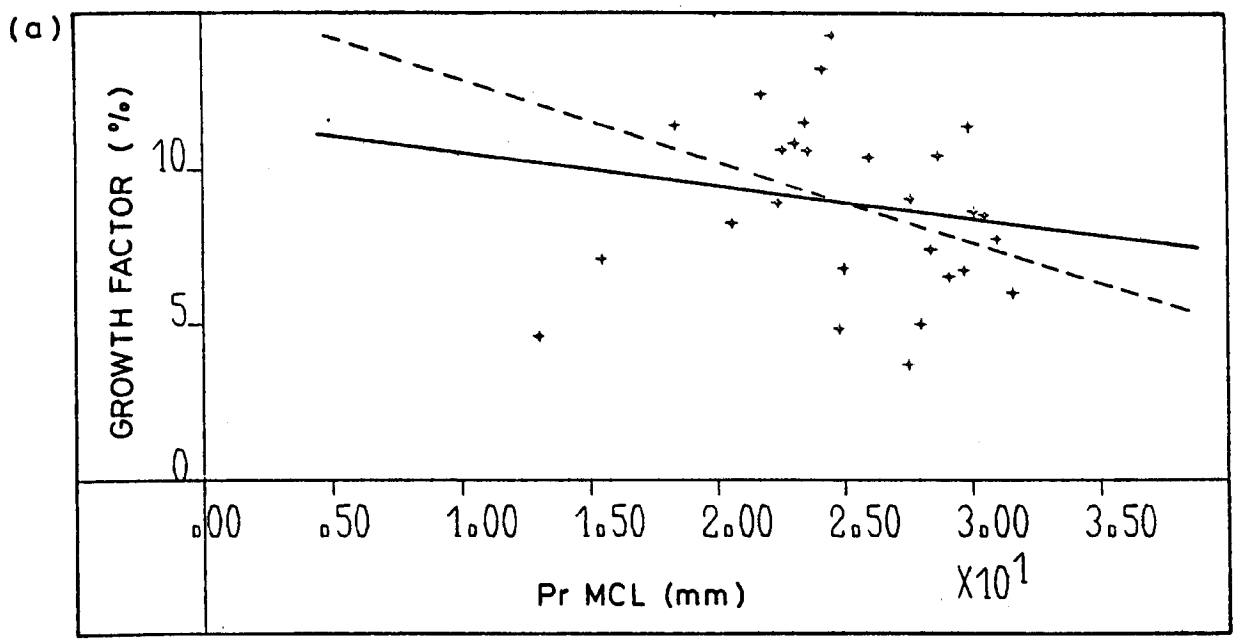


FIG. 5.2 a-d

These computer drawn figures show growth factors (the MI expressed as a percentage of the PrMCL) plotted against the premoult carapace length (PrMCL) for the River Leen (males, a, females, b) and Markfield Quarry (males, c, females, d) populations. The lines fitted are the best fit linear regression lines although only the analysis of the River Leen females proves to be statistically significant. The broken line in each figure is that of the opposite sex for that population, transposed for means of comparison.

FIG. 5.2a-b, RIVER LEEN POPULATION, MALES (a), FEMALES (b)



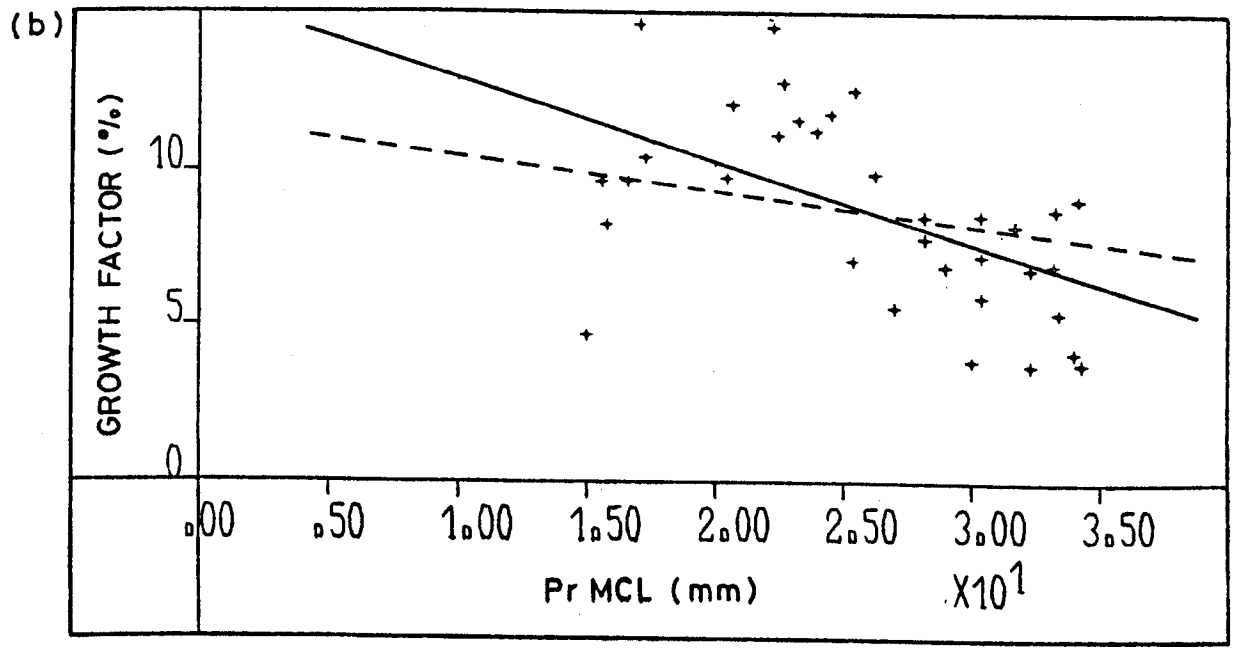
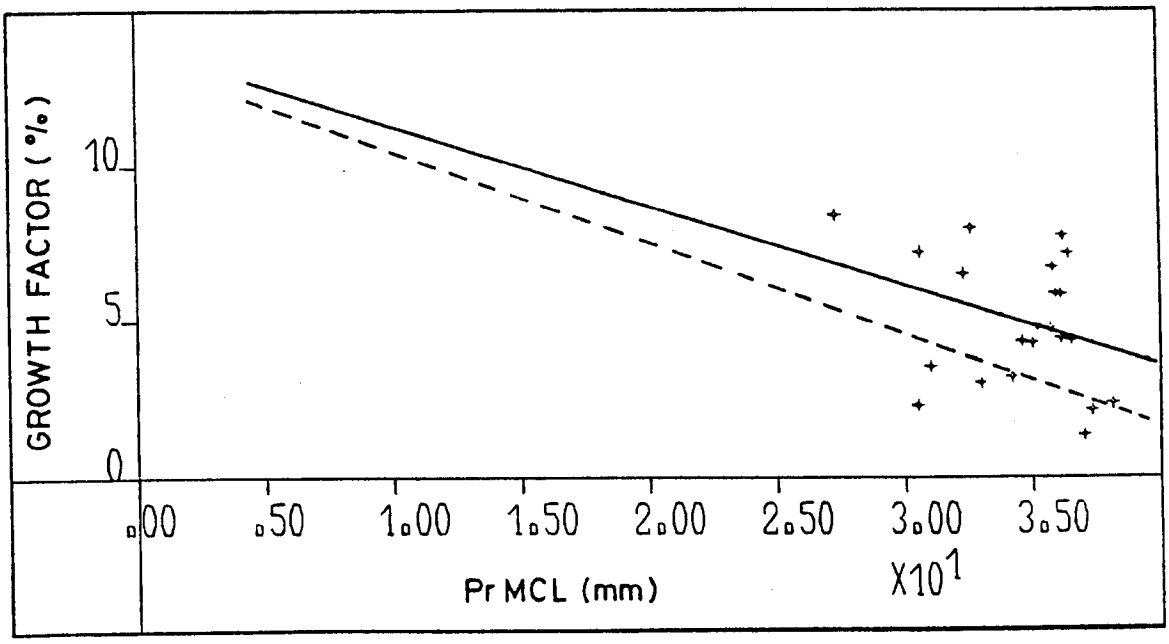


FIG. 5.2c-d MARKFIELD QUARRY POPULATION, MALES (c), FEMALES (d)

(c)



(d)

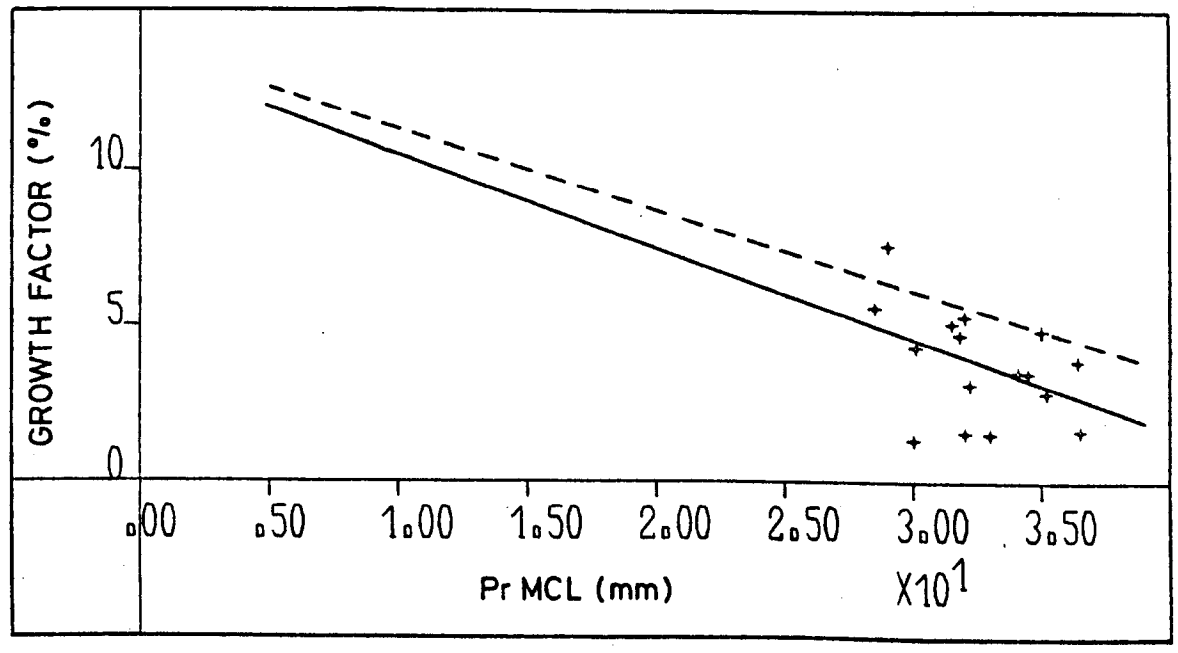


FIG. 5.3a-d AND 5.4a-d

5.3; These computer drawn figures illustrate the Hiatt-growth diagram i.e. the postmoult carapace length (PoMCL) plotted against the premoult carapace length (PrMCL). The curve fitted to the points by the computer is only very slight. Nevertheless a logarithmic transformation of the data is seen to produce the most reliable model of the raw data (in all cases except the Markfield females (5.3d)) when a regression analysis is conducted. The log. transformed data is illustrated in fig. 5.4a-d, to which the best fit regression line has been applied. The broken lines relate to the opposite sex of the same population. Details of the regressions conducted may be found in Table 5.3.

FIG. 5.3a RIVER LEEN MALES, HIATT-GROWTH-DIAGRAM

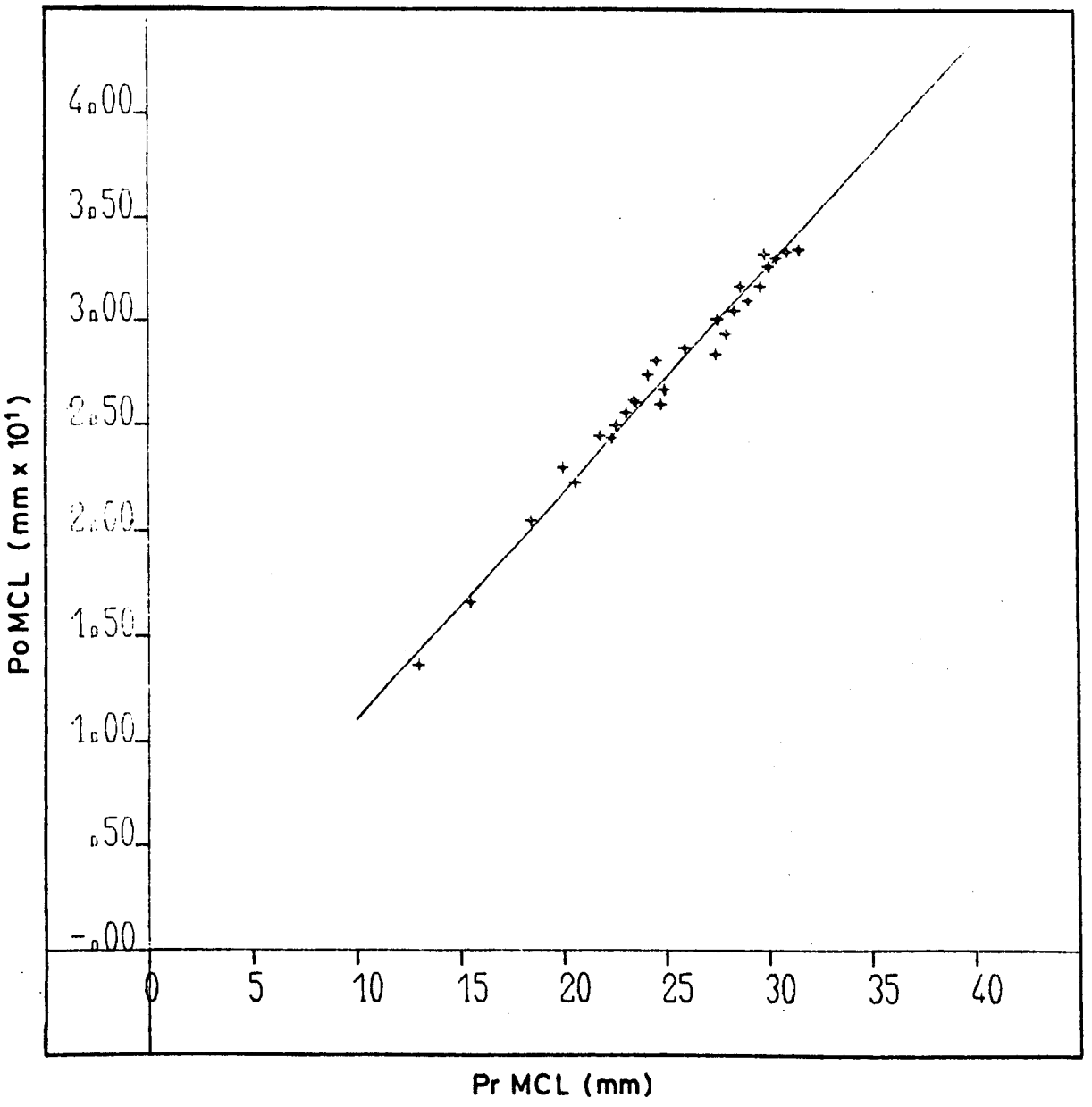


FIG. 5.3b RIVER LEEN FEMALES, HIATT-GROWTH-DIAGRAM

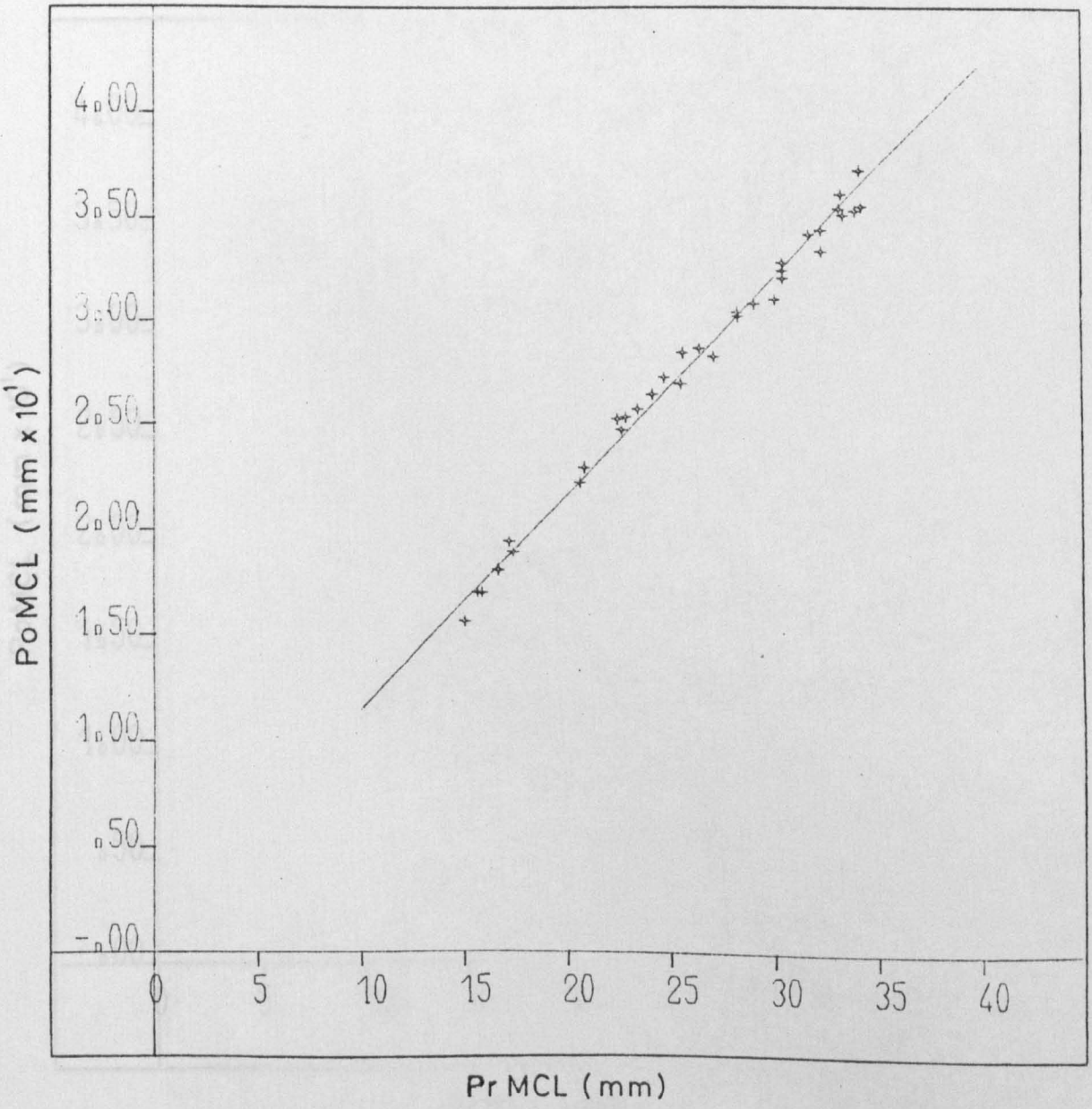


FIG. 5.3c MARKFIELD QUARRY MALES, HIATT-GROWTH-DIAGRAM

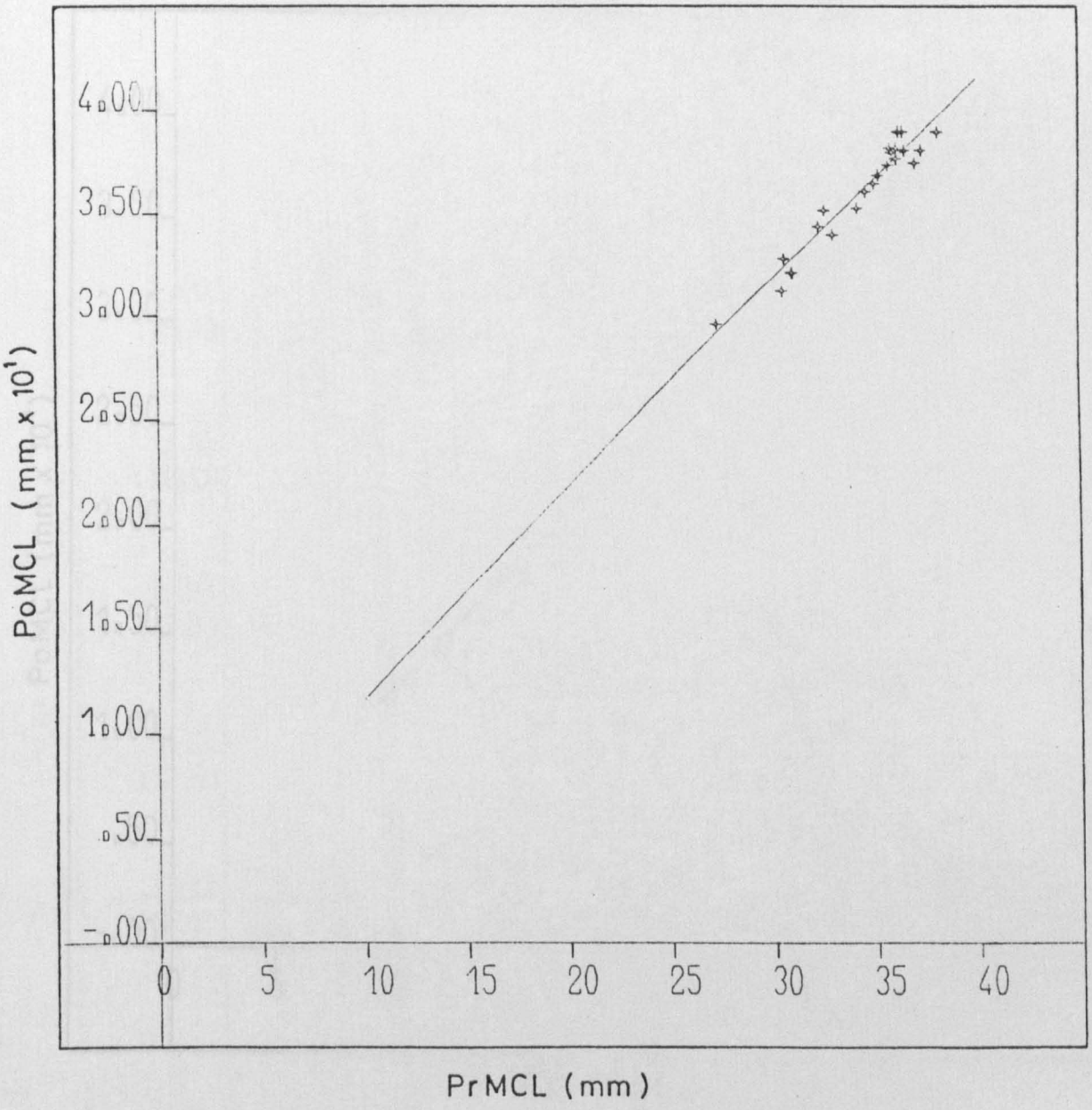


FIG. 5.3d MARKFIELD QUARRY FEMALES, HIATT-GROWTH-DIAGRAM

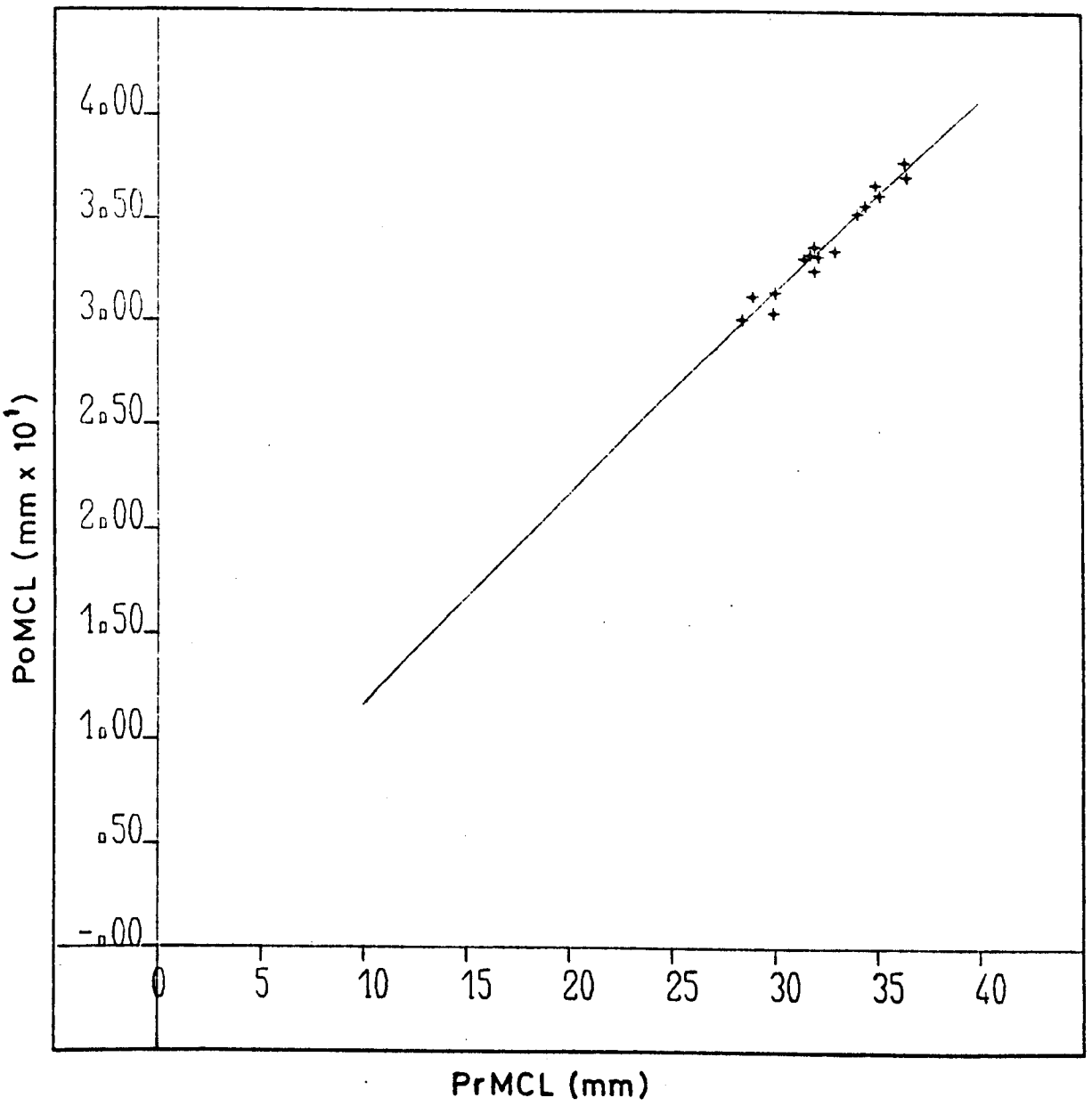


FIG. 5.4a RIVER LEEN MALES, LOG TRANSFORMED HIATT-GROWTH-DIAGRAM

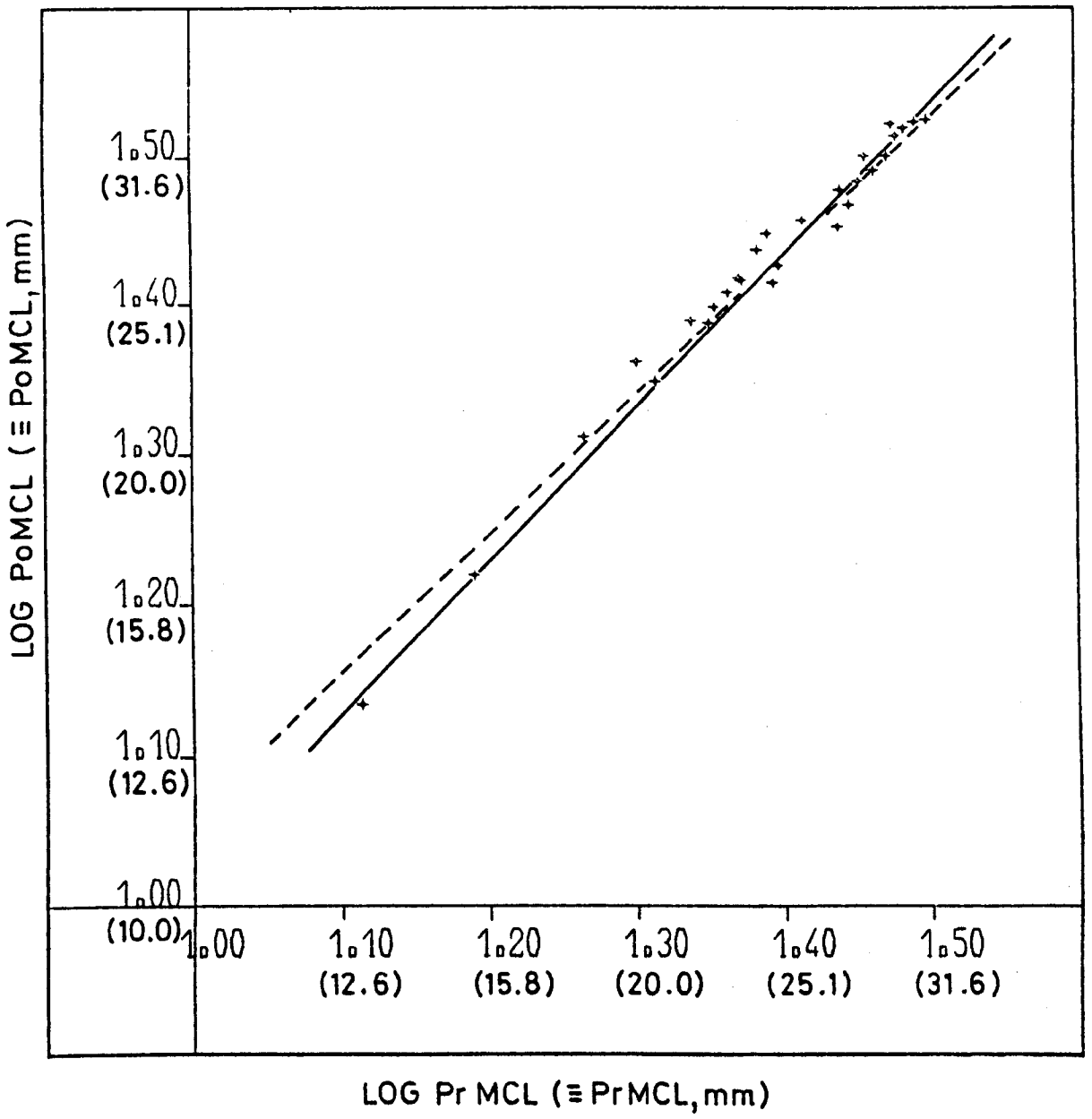


FIG. 5.4b RIVER LEEN FEMALES, LOG. TRANSFORMED HIATT-GROWTH-DIAGRAM

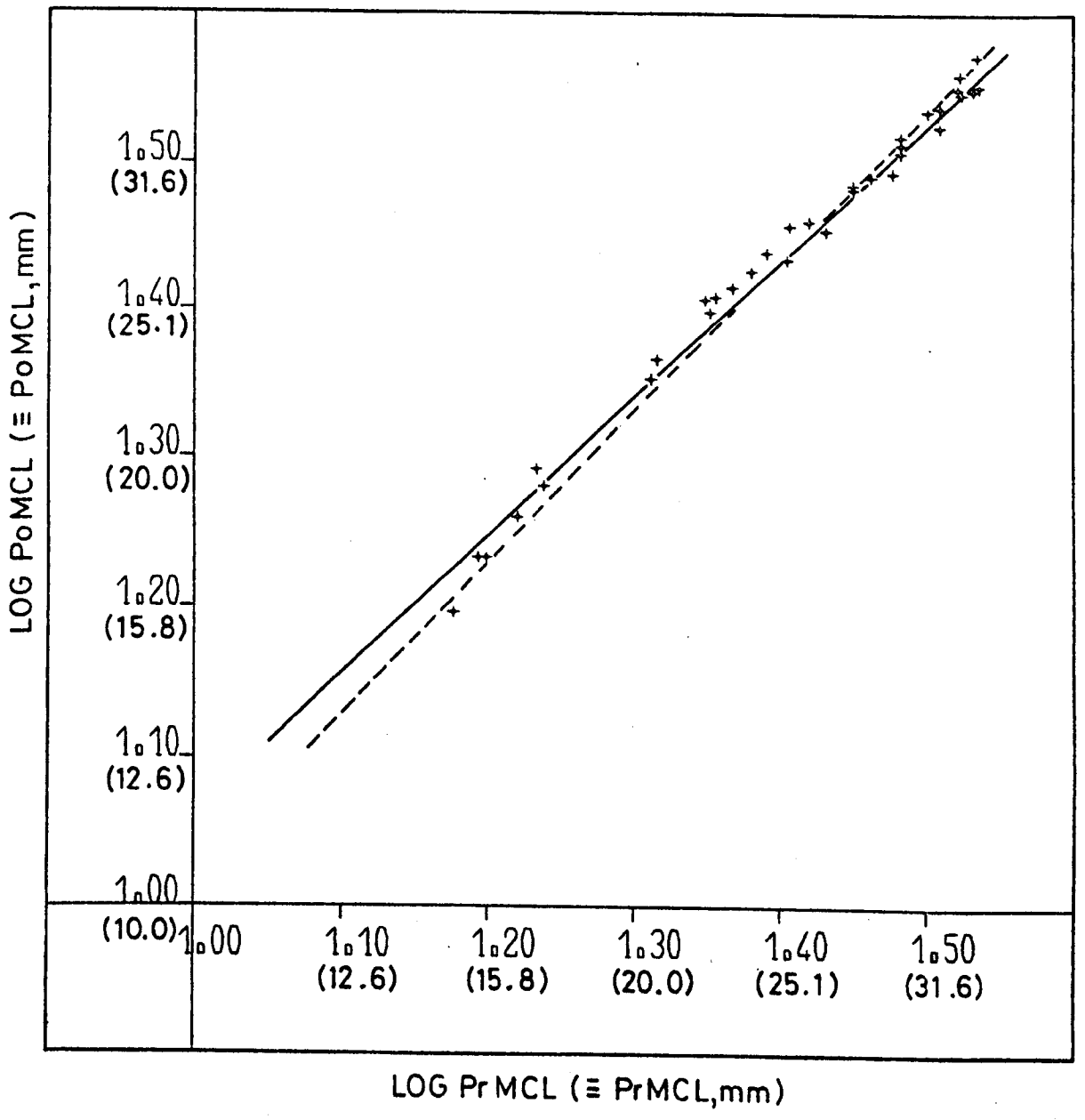


FIG. 5.4c MARKFIELD QUARRY MALES, LOG. TRANSFORMED HIATT-GROWTH-DIAGRAM

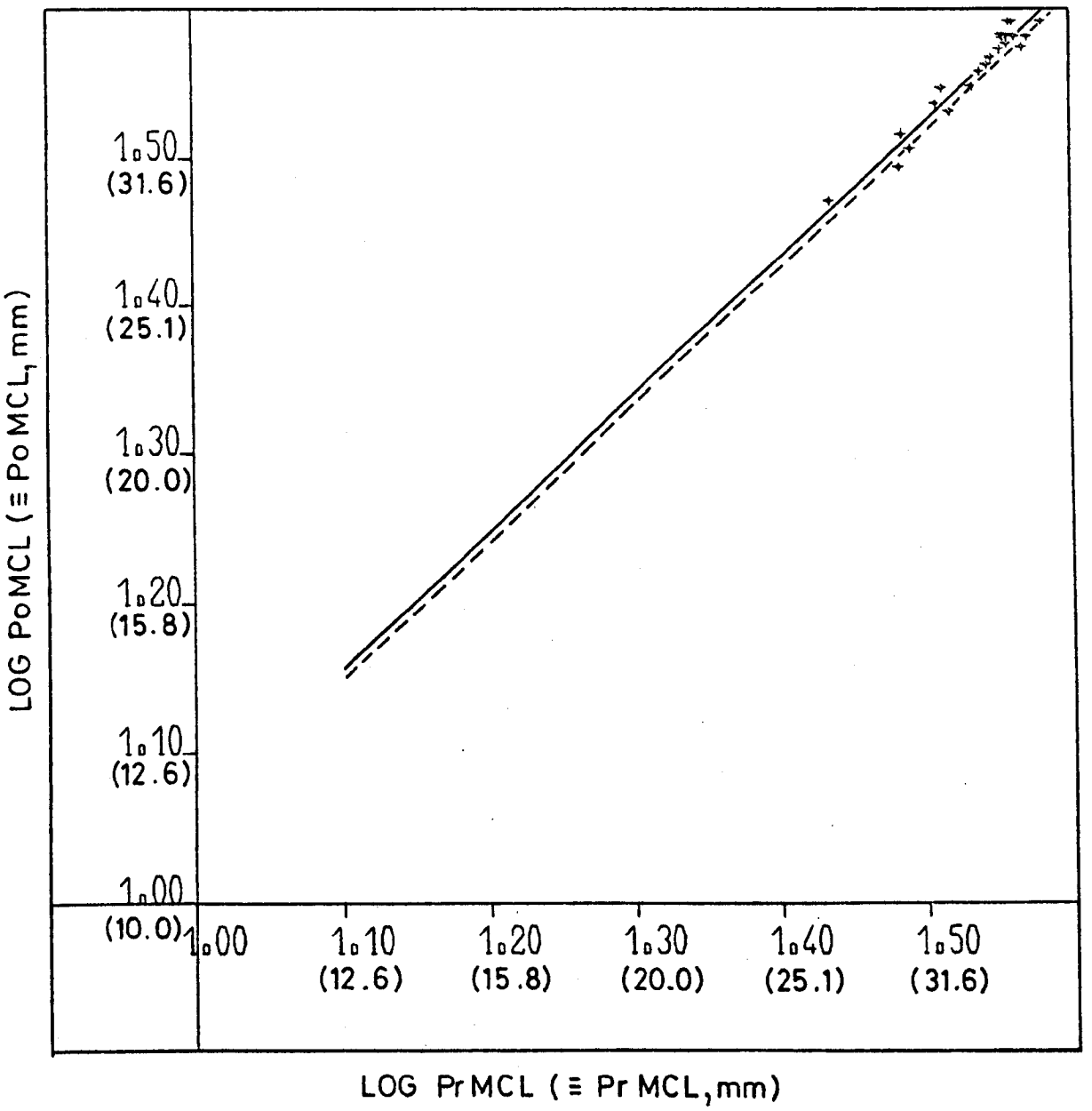


FIG. 5.4d MARKFIELD QUARRY FEMALES, LOG. TRANSFORMED HIATT-GROWTH-DIAGRAM

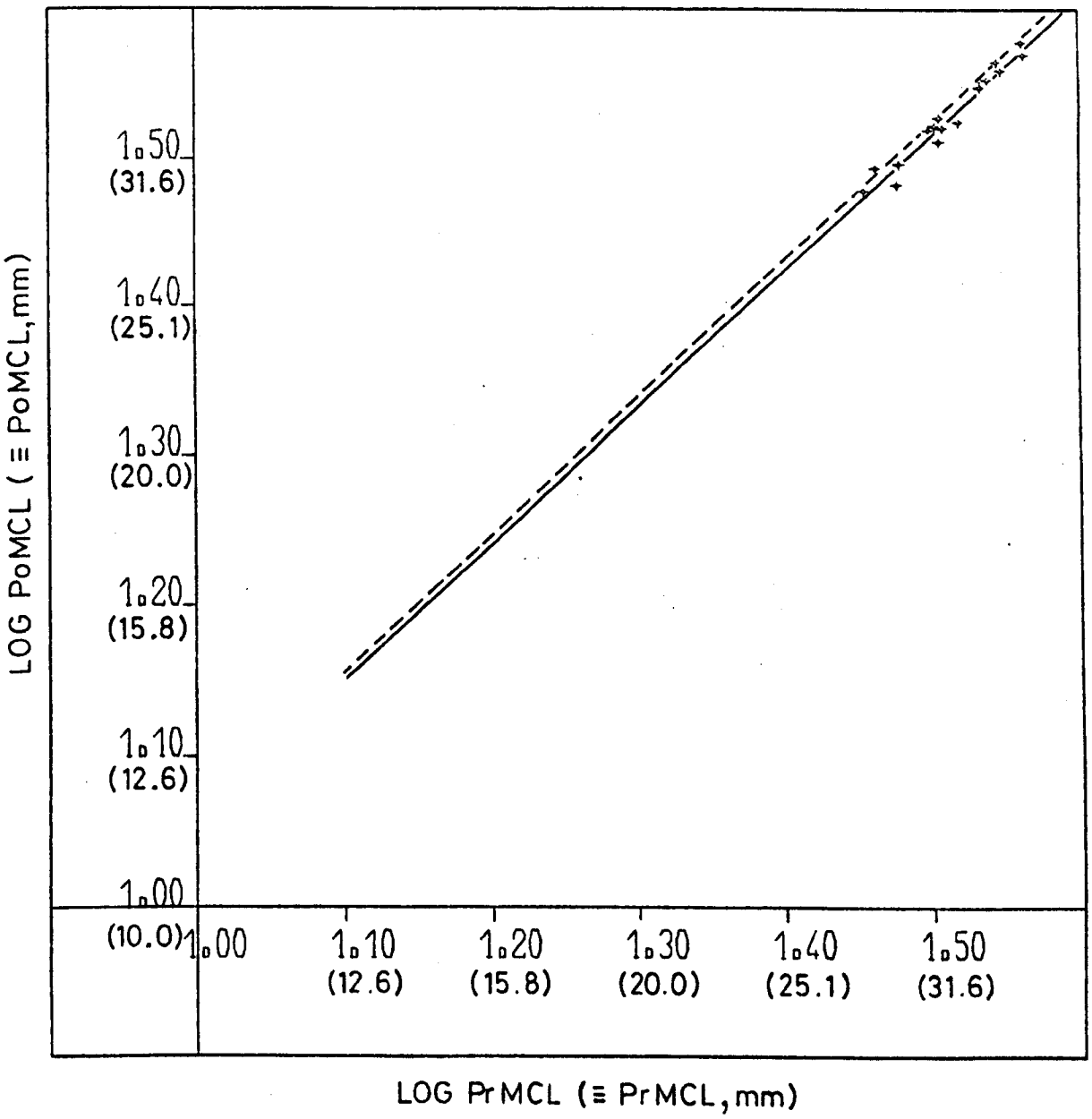


FIG. 5.5a TO SHOW THE SIZE FREQUENCY DISTRIBUTION OF CRAYFISH CAUGHT IN THE RIVER LEEN DURING THE WINTER MONTHS OF 1979/80

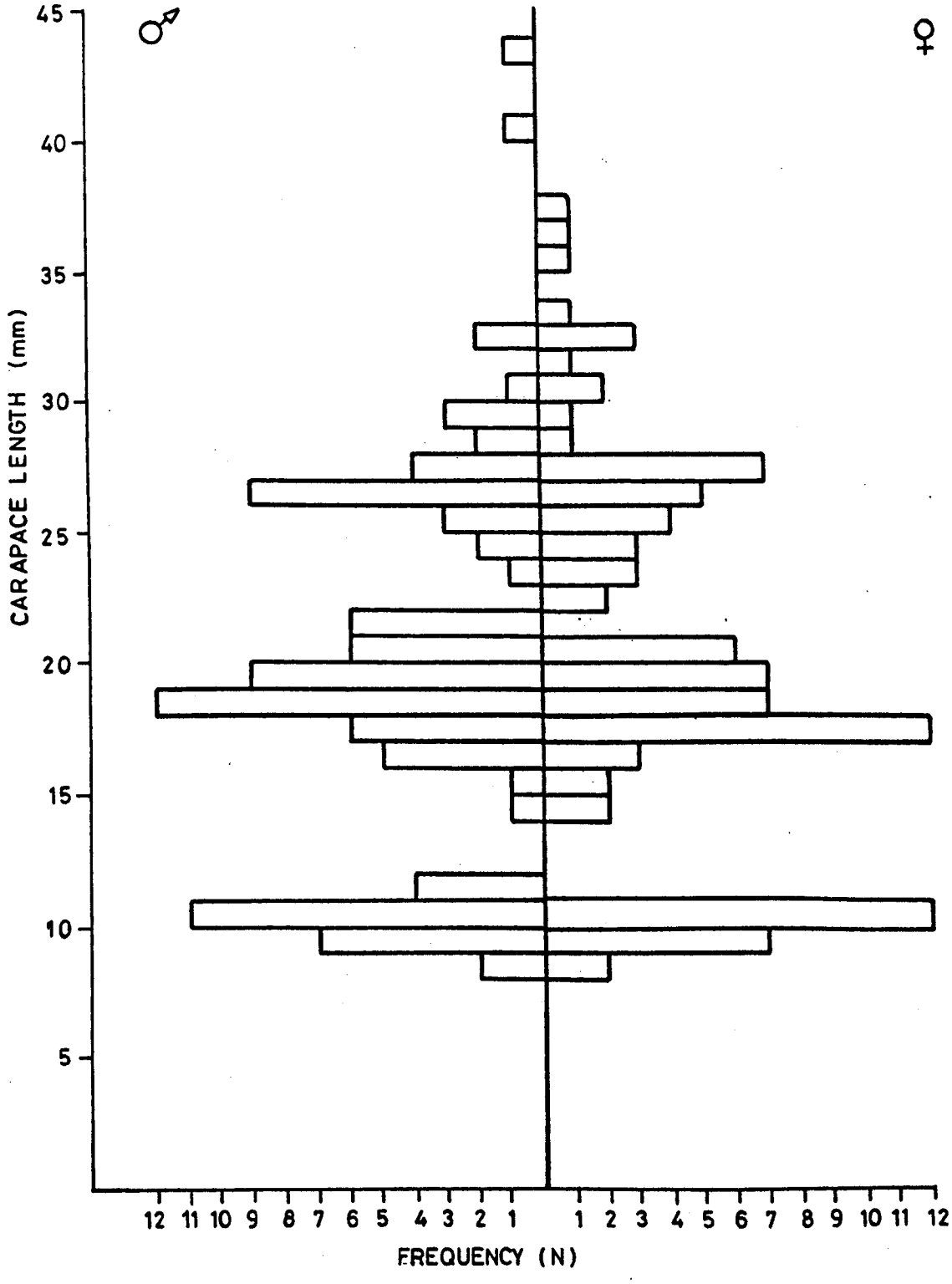


FIG. 5.5b TO SHOW THE SIZE FREQUENCY DISTRIBUTION OF CRAYFISH CAUGHT IN THE RIVER LEEN DURING THE WINTER MONTHS OF 1980/81

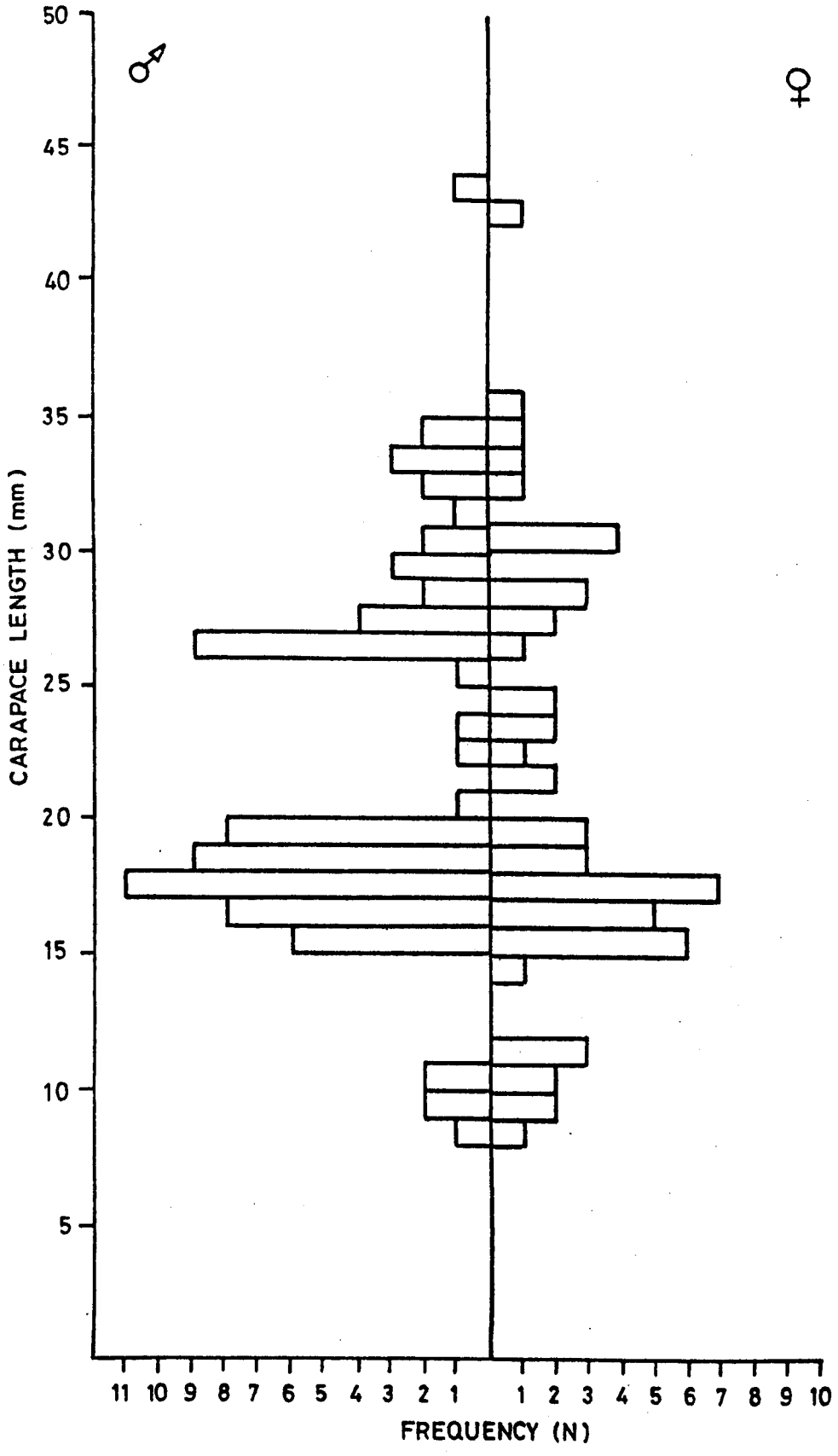
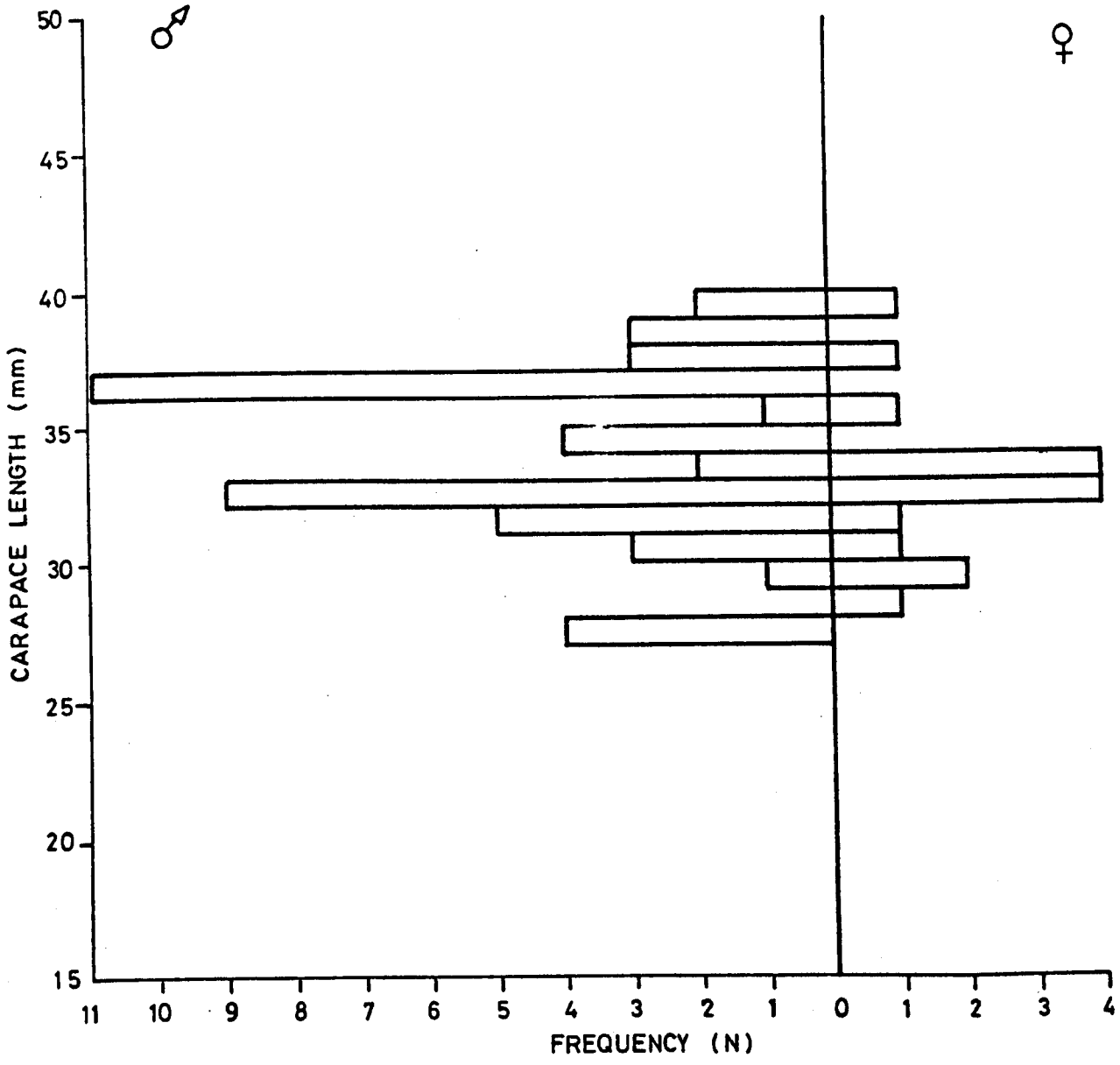


FIG. 5.6 TO SHOW THE SIZE FREQUENCY DISTRIBUTION OF CRAYFISH CAUGHT IN MARKFIELD QUARRY DURING THE WINTER OF 1980/81



FIGS. 5.7a-d AND 5.8a-d

These figures show the polymodal size frequency distribution analysis (see 5.1(ii); Cassie, 1954) of the data given in Figs. 5.5a-b and 5.6. The population and sex being analysed is detailed on each figure.

The solid lines show the probit of the percentage cumulative frequency plotted against carapace length. The arrows indicate the points of inflexion in the line which denote the change from one mode (or age class) to the next. Each mode may then be replotted (broken lines) and the average size of that age class may be read at the point where 50% cumulative frequency cuts the line. The method is detailed in the text (5.1).

FIG. 5.7a RIVER LEEN MALES, WINTER 1979/80

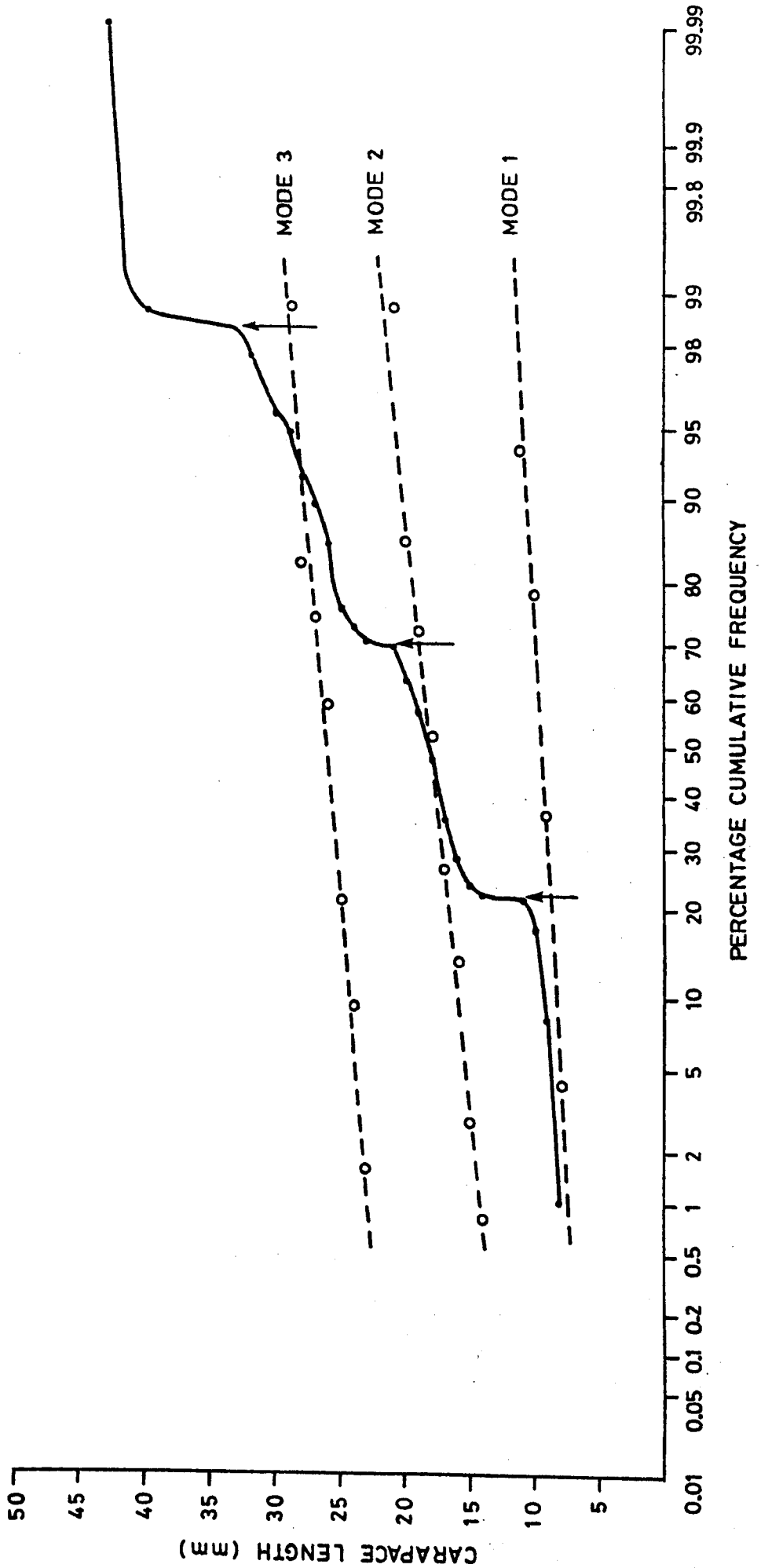


FIG. 5.7b RIVER LEEN FEMALES, WINTER 1979/80

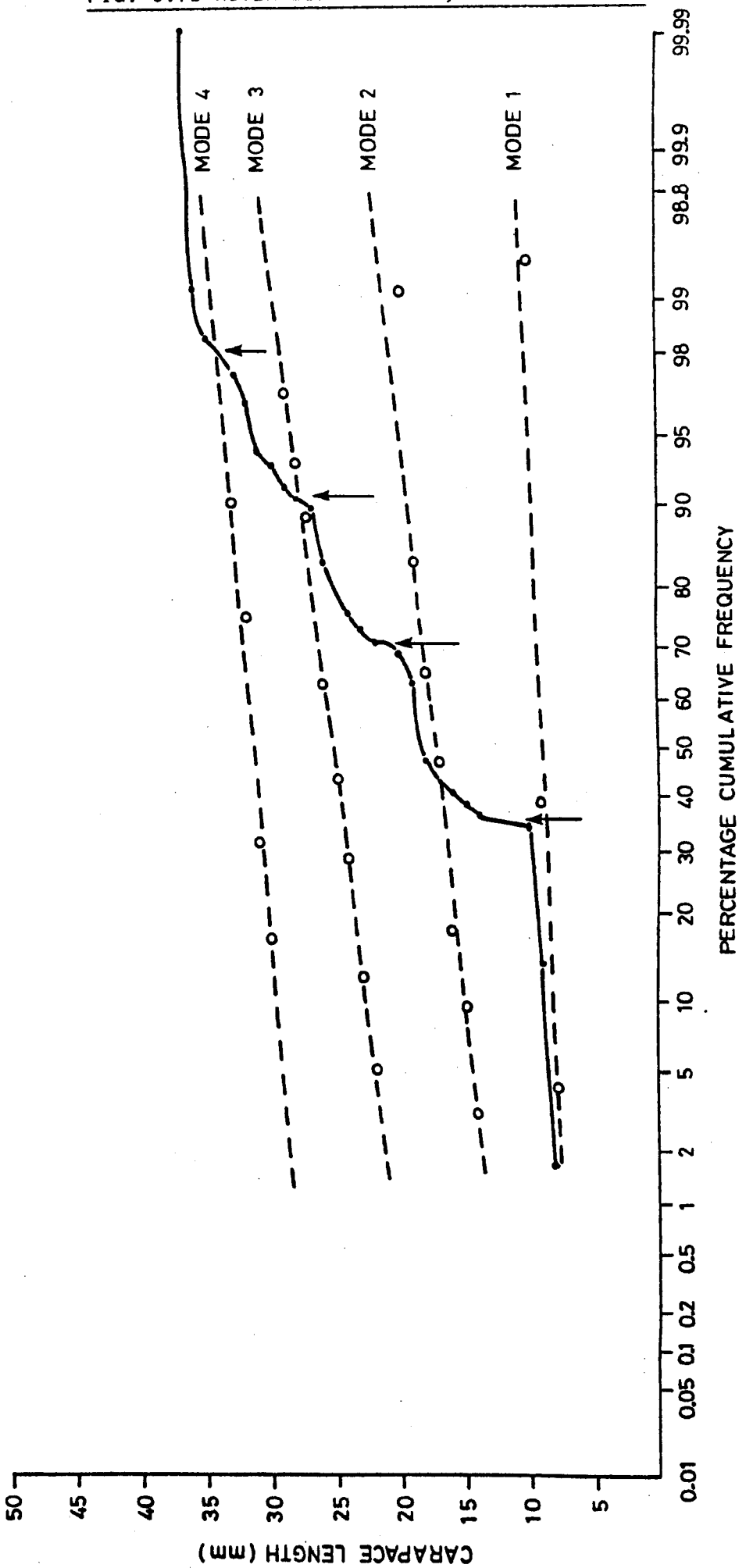


FIG. 5.7c RIVER LEEN MALES, WINTER 1980/81

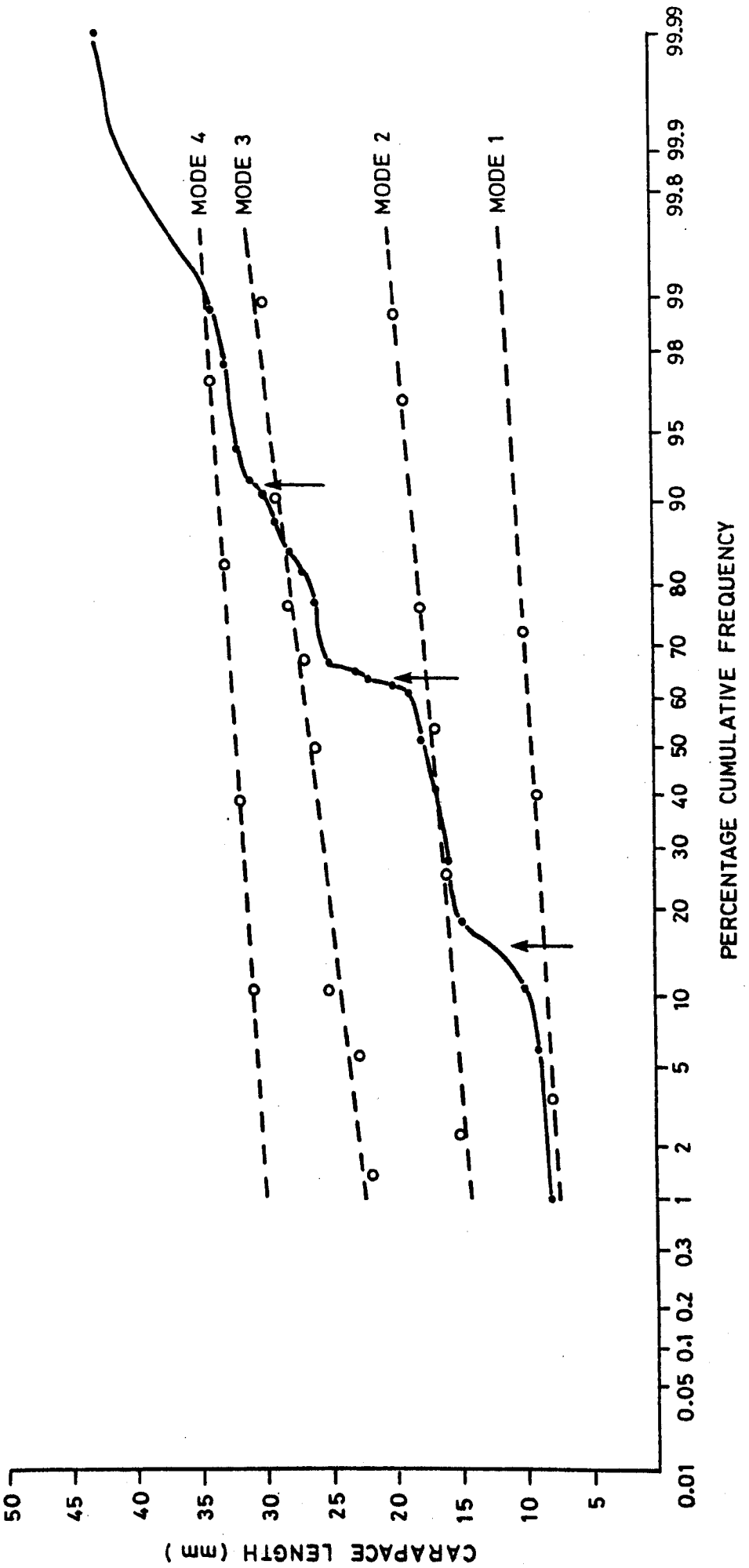


FIG. 5.7d RIVER LEEN FEMALES, WINTER 1980/81

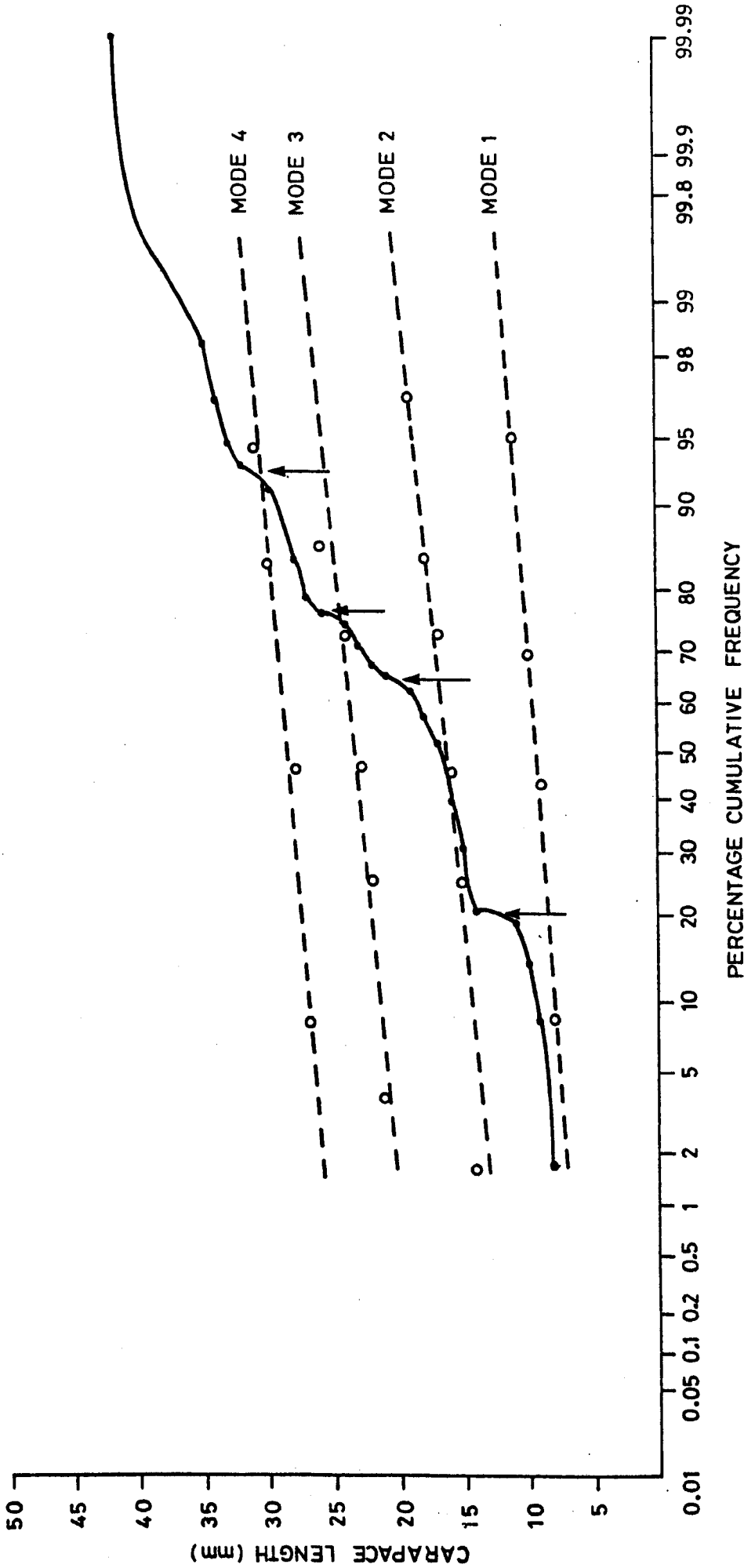


FIG. 5.8a MARKFIELD QUARRY MALES, WINTER 1980/81

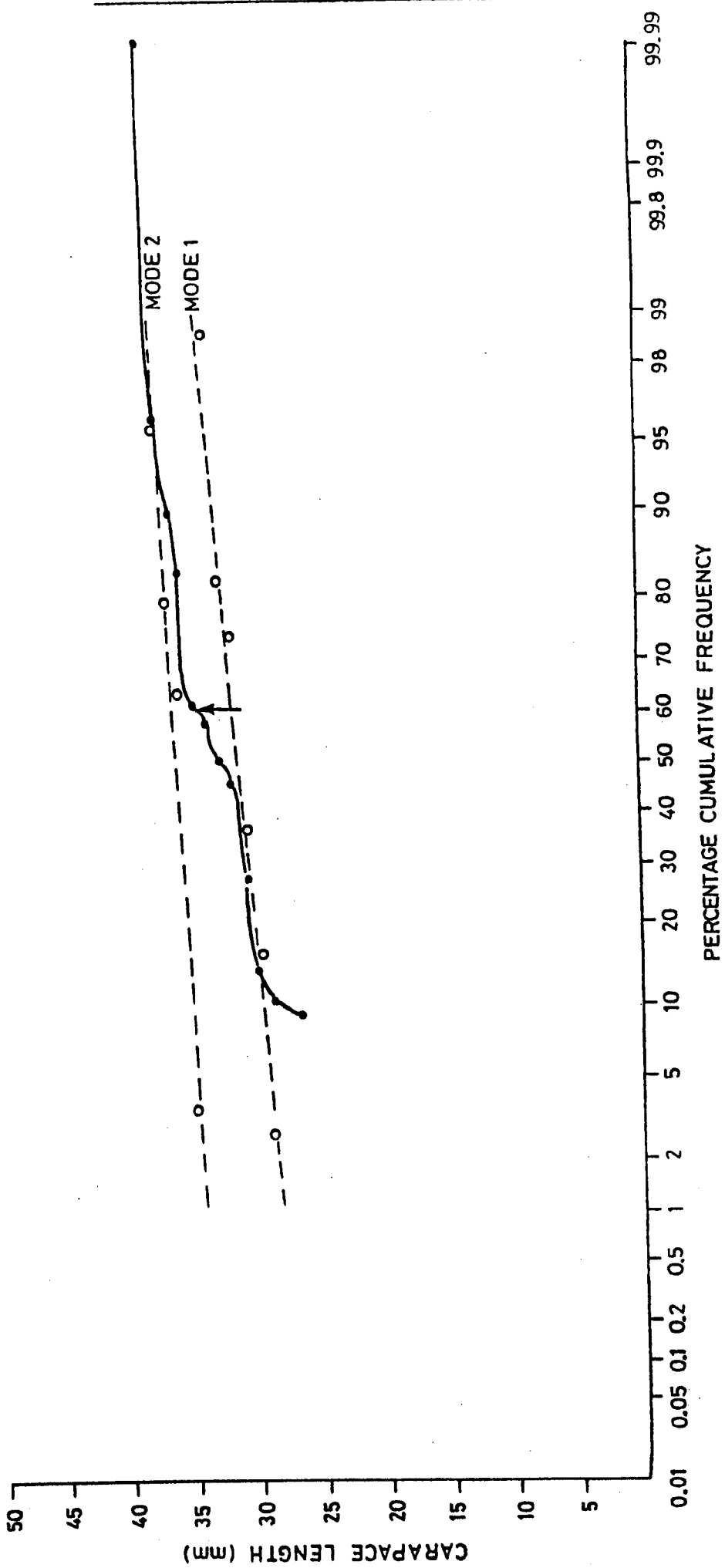


FIG. 5.8b MARKFIELD QUARRY FEMALES, WINTER 1980/81

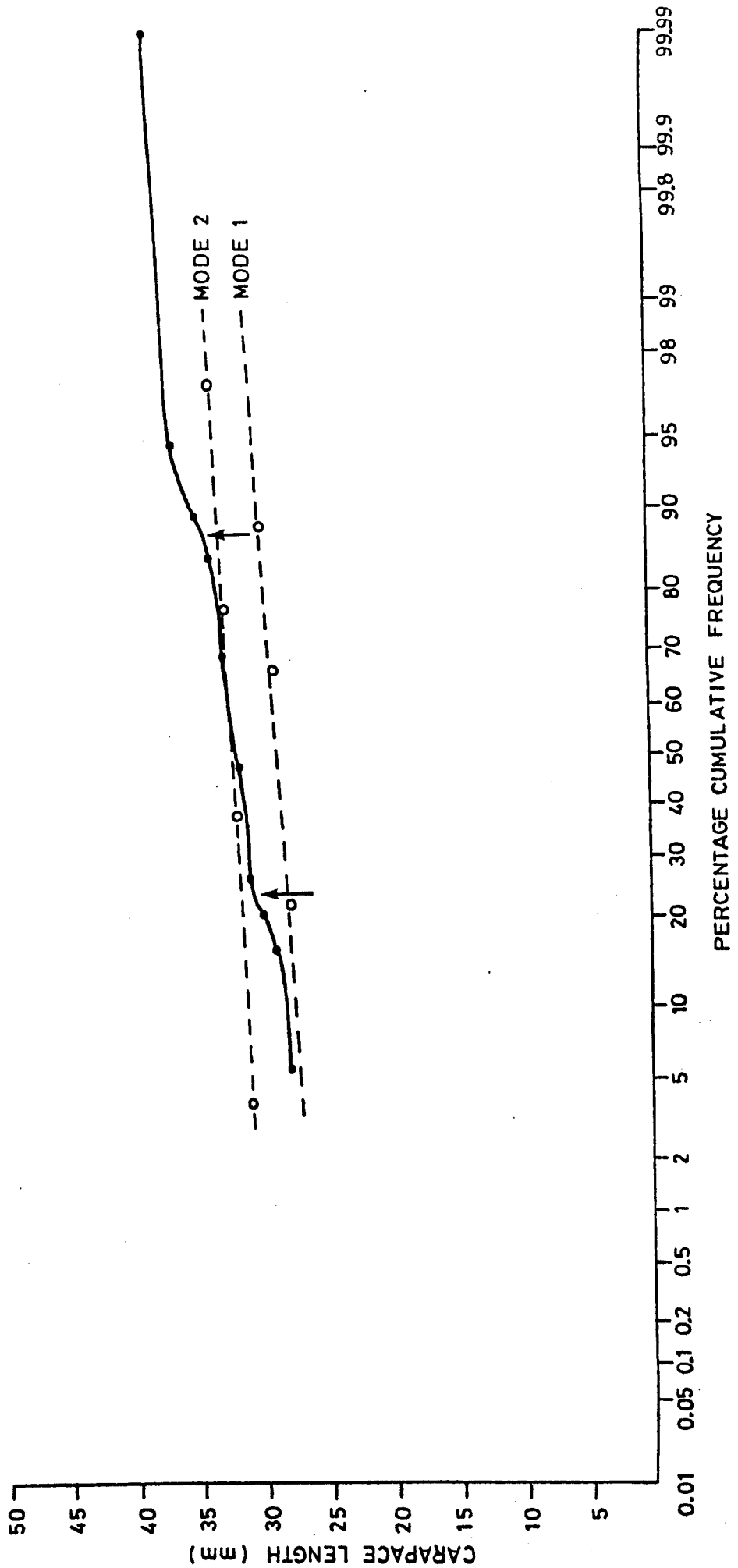


FIG. 5.9a THE GROWTH OF RIVER LEEN MALES AS ILLUSTRATED BY POLYMODAL SIZE FREQUENCY ANALYSIS FOR EACH MONTH SHOWING 5 YEAR CLASSES (0+→4+) AND ±1S.D.

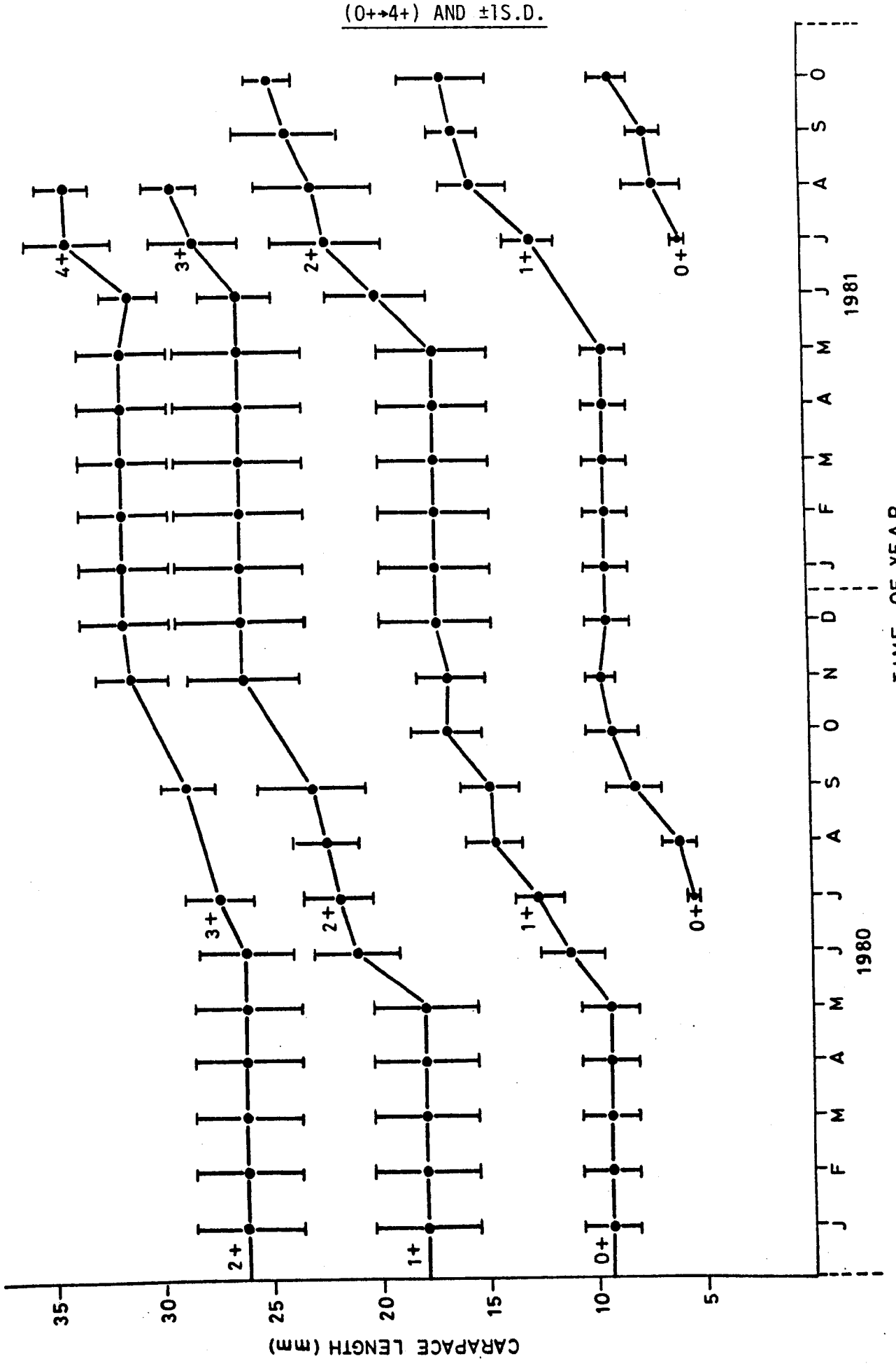
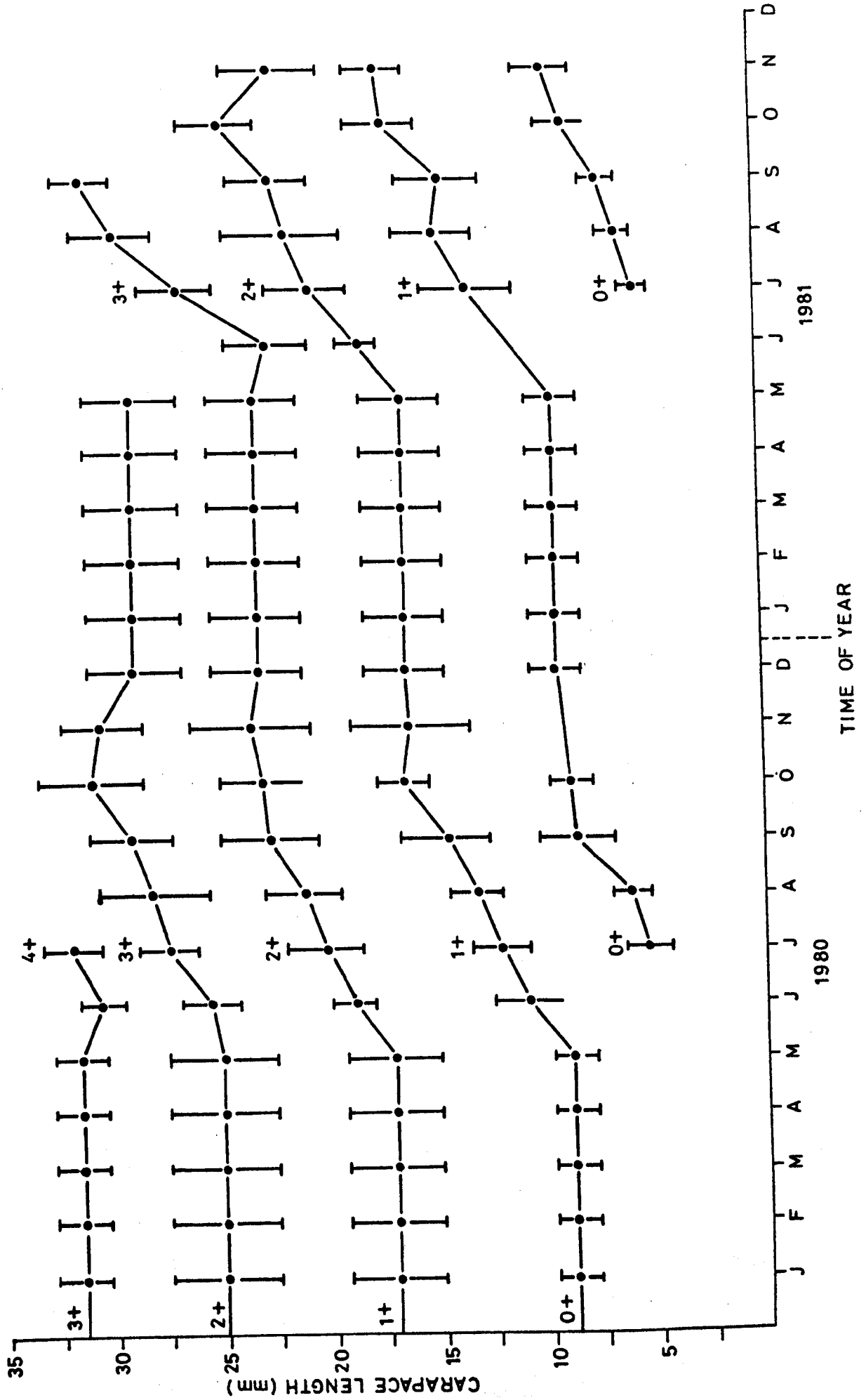


FIG. 5.9b THE GROWTH OF RIVER LEEN FEMALES AS ILLUSTRATED BY POLYMODAL SIZE FREQUENCY ANALYSIS FOR EACH MONTH SHOWING 4 YEAR CLASSES (0+→3+) AND, ±1 S.D.



5.2 THE RELATIVE GROWTH OF *A. PALLIPES* IN THE MIDLANDS

5.2(i) INTRODUCTION

Relative growth refers to the fact that increases in certain dimensions of an animal may occur at different rates from others. The Crustacea demonstrate this phenomenon very well due to the ease of measurement of the hard integument (Hartnoll, 1976), and several studies of relative growth in crayfish have indeed been conducted (e.g. Kossakowski, 1962, 1967; Nefedov and Mazanov, 1973; Mason, 1974; Flint, 1975; Lindqvist and Louekari, 1975; Romaire *et. al.*, 1976; Rhodes and Holdich, 1979).

As early as 1924 it was demonstrated that most examples of relative growth may be explained by the general equation:

$$Y = Bx^{\alpha}$$ (Huxley, 1924).

This equation implies that the two dimensions grow relative to each other at a constant rate which is defined by α. They show a curvilinear relationship and α refers to the relative growth rate, also known as the level of allometry (Hartnoll, 1976). When;

$\alpha > 1$, then growth shows positive allometry,

$\alpha = 1$, then growth is isometric,

$\alpha < 1$, then growth shows negative allometry.

For ease of analysis, the curve may be 'linearized' by a logarithmic transformation, thus;

$$\text{LOG } Y = \text{LOG } B + \alpha \text{ LOG } x$$

Standard linear regression analysis is now possible, and α is represented by the slope of the line.

The level of allometry may not be constant throughout the entire life history of an animal. α will then change with the

different phases experienced in the life history, for example, from the larval to post-larval stages of the lobster. A separation of the growth phases may also occur around sexual maturity. The puberty moult which occurs at this time is very often distinguishable as an obvious critical moult and is the most prominent feature of post larval growth. Thus when more than one phase occurs, each phase may be plotted as an independent regression line. When it is not possible on morphological grounds to place an individual in either phase, then an inflected line occurs, compared with the discrete lines, which do not intersect, that occur when obvious morphological differences exist at maturity (Hartnoll, 1976).

In certain crayfish species such as *Procambarus*, a critical moult is observed when crayfish change from the non-reproductive form II to the reproductive form I (Romaire, *et. al.*, 1976; Stein, 1977; Payne, 1978), but this does not occur for the Astacinae which include *A. pallipes*. Hence it is not possible to produce two distinct lines, and if different phases of allometric growth do occur for *A. pallipes* then they will be apparent as an inflexion in the Log. transformed linear regression line.

This study compares the allometric growth of various dimensions of *A. pallipes* from three Midlands populations; the River Leen, Markfield Quarry, and Nanpantan Reservoir. Comparisons are made between populations, and between the sexes within each population. The information gained is considered to be of importance in determining appropriate sizes for the exploitation of crayfish as a food resource in that certain of the dimensions examined relate to the potential meat yield (e.g. chela length/width, abdomen width).

5.2 (ii) METHODS

All crayfish collected from each of the study sites and Nanpantan Reservoir were measured accurately to 0.01 mm using Vernier calipers, and weighed to 0.01g as described previously (see 2.4). The dimensions recorded were the total length, carapace length, carapace width, chela length and width, and the length of the moveable digit of the chela, rostrum length and width, telson length and width, the width of the widest abdominal segment and the weight. Males and females were treated separately for analysis, and each dimension was compared against the carapace length which was chosen as the reference dimension. All data accumulated over the entire study period were employed in the analysis, and hence this formed a large size stratified sample for each population concerned. Certain animals were excluded from the analysis, namely those damaged, diseased, or regenerating chelae. The precise numbers of animals employed in each case occur in the tables of results.

Both linear and log. linear regressions were conducted and compared using the criteria described in 5.1. The best fit lines from each regression were compared using the t-statistic previously described (5.1). Comparisons were made between the populations and between the sexes within a population. Where allometric growth was seen to occur (i.e. the log-transformed data produced the most significant regression) a series of regression analyses were conducted about a range of carapace lengths in 1 mm steps, and the combination resulting in the least sums of squares of the residuals indicated the inflexion point of the line. Analysis of the data was effected using the SPSS package on the Nottingham

University ICL 2900 computer.

5.2(iii) RESULTS

Only the best fit regression data are presented although examples of the alternative analysis are given. The equations of the regression analysis are presented in Tables 5.9 - 5.11. A linear description of the data occurs when there is isometric growth. In certain cases it was debatable on the evidence of the values of r, F, and the plots of the residuals, whether the linear or log. linear lines provided the best fit. This is to be expected since the linear line is more accurate due to the introduction of a further constant in the description of the line, which is not included in the equation of the curve.

The equation of a straight line is:

$$Y = a + bx$$

and a more complete description of a curve than that given in the introduction is:

$$Y = a + bx^{\alpha},$$

where 'a' is a constant. It is not included in the log. linear analyses as it would confuse the calculation further. However, in the equation of the curve, α is equal to unity when isometric growth occurs (see introduction), and so the equation for the curve becomes identical to that of a straight line. Consequently, when isometric growth was indicated and it was debatable which line to choose, then the standard linear regression was chosen. The following example illustrates this point; increase in size of abdominal width of male crayfish was seen to represent isometric growth against the carapace length, and both linear and log. linear regressions proved to be highly significant. The analyses of the data were;

POPULATION	VARI-ABLE (x)	REF. DIM. (Y)	EQUATION	r	F	P
LEEN	ASG2	CARL	LOG.Y = 1.0684 LOG.x -0.4236	0.9899	6830.2	<0.001
			Y = 0.4934x -0.5466	0.9890	6296.2	< 0.001
MARKFIELD	ASG2	CARL	LOG.Y = 0.8522 LOG.x -0.1291	0.8237	306.0	< 0.001
			Y = 0.3875x + 1.8627	0.8732	469.5	< 0.001
NANPANTAN	ASG2	CAR	LOG.Y = 1.0377 LOG.x -0.3905	0.9901	1298.8	< 0.001
			Y = 0.4732x -0.2626	0.9903	1370.1	< 0.001

It may been seen that α is almost unity for both the Leen (1.07) and Nanpantan (1.04) populations, and that in each case the values for r and F for both the linear and log. linear analyses are virtually identical. They would tend to indicate that the log. linear line is the best fit for the Leen data, and that the standard linear regression was the most suitable for Nanpantan. The data for Markfield Quarry do not fit into the argument so well in that the value for α is less than unity (0.85) and so would tend to indicate negative allometry. However, the values of r and F indicate that the linear regression is a better fit than the log. linear analysis, and so implies isometric growth. Thus, since each population differs it is most convenient to employ the standard linear analysis in each case, in order that direct comparisons may be made between the populations. Also, as discussed above, when isometric growth occurs, the linear analysis is the more correct. In this particular example growth is seen to be isometric for the males but positively allometric for the females, and consequently inter-sex comparisons are conducted using the log. linear transformed data.

When allometric growth occurs the log. linear regression will always produce the best fit. This is well illustrated by considering the data for the chelae length vs carapace length of the Leen males. The two analyses conducted on this data give the following results.

VARIABLE (x)	REF. DIM. (Y)	EQUATION	r	F	P
CHELA LENGTH	CARAPACE LENGTH	LOG.Y = 1.2739 LOG.x -0.5544	0.9800	13,922.7	< 0.001
		Y = 0.9445x -6.0306	0.8922	2,520.6	< 0.001

Both produce highly significant results. However, examination of the plots of the standardized residuals against the predicted standardized dependent variable clearly reveals that the predicted linear regression line fits very poorly to the raw data (see Fig. 5.10). Quite clearly a curve is implicated as being the true nature of the raw data. Consequently the residual plots of the log. transformed data show that this analysis is far superior. The residuals are seen to be even about the predicted line, but variation is greater as the dependent variable increases (see Fig. 5.11). Evidence that the log. transformed data provides the best fit is also apparent from consideration of the r and F values. Indeed, the F value produces a statistic 5.5 times greater for the log. transformed data than for the linear data. Fig. 5.12 is a computer drawn representation of the raw data drawn with linear axes. The curve produced by plotting the chela length against the carapace length is quite apparent, and indicates the curvilinear relationship expressed between these two variables,

with the former showing positive allometric growth with respect to the reference, dimension, the carapace length.

The above examples illustrate the method of analysis applied to all of the data, and how the pattern of growth was determined to be either allometric or isometric. Positive allometric growth is found to occur for both males and females of each population for the chelae length, and the weight of crayfish, when compared against the carapace length. It also occurs for the abdomen width of females, but not males of each population. These analyses are taken further to assess whether an inflexion point in the line occurs thus indicating a puberty moult (see Introduction). All other dimensions show isometric growth indicating that they are growing at the same rate relative to the reference dimension. It is possible to compare these results without further analysis. The results are expressed in Figs. 5.13 - 5.15.

In addition, regression analyses are also conducted for the chela width and length of the moveable digit against the chela length. Both show isometric growth (see Tables 5.9 - 5.11) indicating that they too show an allometric relationship to the carapace length, but that the dimensions of the chelae themselves change isometrically to each other throughout the period of allometric growth. Similarly, isometric growth occurs for the dimensions of the telson (telson width vs telson length) and the rostrum (rostrum width vs rostrum length). These results are expressed in Figures 5.16 - 5.18.

Analysis of the log. transformed data by comparison of the sums of squares of the residual errors of serial regressions reveals that inflexion points occur in the lines of certain variables

at the carapace lengths indicated in Table 5.13. The serial regressions for the log. transformed data are only conducted between 20 mm and 36 mm (C.L.) since if a puberty moult occurs, then it should be identified within this range. The linear regressions conducted by this author are discussed later, but from the transformed data it may be seen that if the inflexion point does indeed indicate a puberty moult, then based on the analysis of male chelae, sexual maturity of male crayfish occurs in the River Leen, Markfield Quarry, and Nanpantan Reservoir at 21 mm, 30 mm and ≥36 mm (C.L.) respectively! This seems highly improbable, and it is suggested that these inflexion points are anomalies caused by each set of data (e.g. see also 5.1; also Mauchline, 1976). They do indeed occur, but they are not consistent with the argument that they represent a puberty moult.

Since the inflexion points represent statistical anomalies rather than physiological events in the life history of the crayfish, bearing no relationship to the sizes ascertained for sexual maturity previously (3.2), it was decided to discard these findings in any further discussion. However, it is certainly true that the level of allometry is changing constantly. Consequently it was decided to analyse all of the dimensions showing allometric growth about an inflexion point of 25 mm (C.L.), the approximate size at which sexual maturity occurs. This, it must be stressed, is not a true inflexion point of the type referred to in the introduction to this section (Hartnoll, 1976), and it may not be said that a puberty moult is apparent for *A. pallipes*. However, it is of value to analyse the results about 25 mm (C.L.), the known size of sexual maturity, since such a representation of

the data will more accurately describe the growth of different size classes of the population as the level of allometry gradually changes.

The equations of the lines plus and minus 25 mm (C.L.) are given in Table 5.14 for each of the allometric variables, viz. Chela length, weight, and the second abdominal segment. The slopes of the regressions, equivalent to the level of allometry, α, are compared using the t-statistic. Comparisons are made for the lines within each population which represented the situation $\pm$ maturity (25 mm C.L.), between each sex within each population at $\pm$ maturity, and between each population for each sex at $\pm$ maturity. The results are expressed in Tables 5.15(i)-(iii) and Figs. 5.19 - 5.22.

Study of the results given in Tables 5.9 - 5.11 and 5.14 reveals (on consideration of the F and P values for all analyses conducted for both isometric and allometric dimensions) that the predicted lines show a high degree of significance and correlate well with the raw data. Table 5.16 provides a more easily digestable summary of all of the analyses and comparisons made. Sexual dimorphism is seen to occur for several of the variables. The chelae of males from each population are significantly larger than those of the females, and show allometric growth in both the mature and immature phases. By contrast the chelae of the females show isometric growth ($\alpha \simeq 1$) in the immature phase, changing to allometric growth after maturity. The data for the Nanpantan females is an exception, but it is based on a smaller sample and so the result achieved, which suggests allometric growth in the immature phase, may not be significant. For each population,

comparison of the level of allometry ± maturity reveals that it is significantly greater after maturity. The dimensions of the chelae themselves, i.e. the chela width, and the length of the moveable digit, grow isometrically to the chela length in each case studied. Thus they maintain the same relative proportions to one another throughout the entire life history. Regarding the width of the chelae, sexual dimorphism is seen to occur only for the Leen population. The chelae of the males are wider than those of the females with chelae of the same length. Sexual dimorphism occurs in all populations for the length of the moveable digit, and it is greater for the males of the Leen population, but greater for the females of the Nanpantan and Markfield populations.

The abdomen width shows allometric growth for mature females of each population whilst isometric growth is found to occur for immature females and all sizes of males. Comparison of the level of allometry ± maturity reveals that significantly greater relative growth occurs for the abdomens of mature females (except Nanpantan where sample size was small, precluding direct comparison with the Leen and Markfield). Accordingly, no sexual dimorphism exists for immature Leen animals but does for mature animals. Both immature and mature Markfield animals show sexual dimorphism with respect to this variable.

All phases of growth show an allometric relationship when considering the weight. No significant differences exist for the level of allometry of mature and immature Leen males or Markfield females. However, mature males from Markfield Quarry show a greater level of allometry than immature males and it is also greater for immature Leen females than males. Sexual dimorphism is only

seen to occur between mature animals of the Leen population.

The total length of the animals and the telson length both show isometric growth for each sex, and sexual dimorphism occurs, with females of each population having the greater dimensions. The carapace width changes isometrically to the carapace length, and sexual dimorphism is only seen to occur for the Leen population, the males of which have the greater dimensions. The telson width changes isometrically to its length and although it is seen to be longer for females, it is in fact wider for the males. Sexual dimorphism occurs for this variable for both the Leen and Nanpantan populations, but not Markfield. Isometric growth also occurs for the rostrum length, and its width increases isometrically to its length. No sexual dimorphism occurs for either variable.

Comparison of the populations reveal no consistent differences, and one variable may be greater for the Leen population, for example, but for another variable this population may have the smallest dimensions. Certain notable differences, however, are apparent. The chelae of the males are found to be longer for animals from Markfield and Nanpantan than the Leen, for both mature and immature animals. However, animals from the Leen have significantly wider chelae than Nanpantan, and those from Markfield Quarry are the least wide. No differences exist between the populations regarding the length of the moveable digit. Considering the females, the length of the chelae are similar for Leen and Nanpantan animals, but are greater than those of Markfield Quarry for mature animals. For immature animals Nanpantan Reservoir animals have significantly greater chelae than Leen animals but neither are significantly different from Markfield Quarry. Again

it is seen that the widest chelae belong to the Leen population, but they have the smallest moveable digit.

Examination of the dimensions of the abdomen reveals that those of the Nanpantan and Leen males are greater than those of Markfield males. For mature females, abdomen widths of animals from the Leen and Markfield Quarry are equal and are greater than those of Nanpantan Reservoir. The reverse is true for immature animals. Considering the weight, no population differences exist for mature males and females but do so for immature animals of each sex, the Leen animals being greater than those from Markfield Quarry.

For the remaining variables it may be seen from the summary in Table 5.16 that inconsistent population differences exist similar to those previously discussed. Further discussion is not felt necessary since, unlike the chelae, abdomen width, and weight, the remaining variables are not of potential commercial (economic) concern. The rostrum, however, should be mentioned (see Discussion). It may be seen that considering both sexes, it appears that the length of this variable is greater for the Markfield Quarry animals than the Leen animals, and those from Nanpantan are intermediate and not significantly different from either population. Similarly, regarding the width of the rostrum Nanpantan animals are intermediate whilst those from the Leen have significantly wider rostrums than Markfield Quarry animals.

5.2(iv) DISCUSSION

Allometric growth occurs for the chelae and weight of males and females, and for the abdomen width of females. All other variables exhibit isometric growth. For those variables which

exhibit allometric growth there was no evidence of a puberty moult. An inflexion point did occur in the lines describing these variables, but it was inconsistent with the argument for a puberty moult, and was thought to be a statistical anomaly. Other authors have been able to define separate regression analyses for mature and immature crayfish since they were working with species for which different morphological forms exist representing mature and immature animals. Stein (1977) examined several variables (dimensions) of *Orconectes propinquus* in this way and drew separate log. linear regression lines for animals in Form I, the sexually reproductive form, and Form II, the non-reproductive form. Similar analyses have been conducted for *Procambarus clarkii* and *P. acutus acutus* (Romaire, *et. al.*, 1976)

The Astacinae do not have morphologically different forms and consequently any differences in allometry which occurred at sexual maturity would be apparent as an inflexion point in the log. linear regression line. This is due to the gradual nature of the change with some animals at a particular size being mature whilst others are not (Hartnoll, 1976). Those authors who have considered relative growth of Astacine species have not tended to analyse their data using log. transformations, although some have (e.g. Mason, 1974; Flint, 1975). The increasing proportional growth of the chelae compared to either length or carapace length has, however, been consistently recognized as a curve for both males and females, with sexual dimorphism generally occurring (e.g. *Astacus astacus*, Abrahamsson, 1966; Kossakowski, 1967, 1971; Lindqvist and Louekari, 1975. *A. leptodactylus*, Kossakowski, 1962, 1967. *Orconectes limosus*, Kossakowski, 1962). Kossakowski

decided to analyse the curve by representing it as a series of inflected regression lines for arbitrarily chosen size classes. This method is acceptable, but as was seen from the plots of the residuals, will not describe the true situation accurately.

Rhodes and Holdich (1979) have also analysed data relating to the chelae length of *A. pallipes*. Their sample was pooled from the three populations studied by this author. Linear regressions were conducted, and an inflexion point, which is described as indicating the maturity moult, was found to occur. Since this did not occur with the log. transformed data, this author similarly analysed his data with linear analyses. The results support the view that the inflexion point is a statistical anomaly and bears no relation to a puberty moult. Only male chelae lengths were analysed in this manner and would tend to indicate that maturity was occurring at 27.0 mm, 31.2 mm, and 40.0 mm for the Leen, Markfield, and Nanpantan populations respectively (Table 5.13). Rhodes and Holdich (1979) found an inflexion point at 29.00 mm, probably as a result of averaging the three populations. Their regression analyses therefore do not indicate diphasic growth as suggested, and a log. linear relationship is far superior. Since very little difference was observed between the sums of squares of the residuals when looking for an inflexion point it may almost be said that growth was in fact monophasic. However, it must be stressed that the situation is not as simple as this. Monophasic growth would imply that the level of allometry (α) was constant. However, it in fact changes gradually throughout the life history and hence it is acceptable to consider the allometric growth as two phases about an arbitrarily chosen point,

in this case, the observed size for sexual maturity, 25 mm (C.L.). In this way it is possible to compare the level of allometry occurring for the different phases of growth.

Other authors working with *A. pallipes* similarly have not realized the full potential of their data. Morriarty (1972) analysed weight against carapace length in a linear fashion when it is clear that this variable should vary with the cube of the chela length (see also, Nefedov and Mazanov, 1973). Brown (1979) recognized that allometric growth was represented by a curve but linearized it by transforming only one variable, producing a relationship of the square root of the chela length against the carapace length for the females, and the log. of the chela length against the carapace length for the males. This author also attempted similar regressions, but found that the log. log. transformed data was superior.

The physiological mechanisms which control allometric growth are not understood, but two models have been proposed, both of which are discussed at some length by Hartnoll (1976). Briefly, they are; the growth increment hypothesis (Mayrat, 1967) which suggests that within any phase the actual amount of growth is regulated and is genetically determined. The second hypothesis is the size equilibrium hypothesis (Huxley, 1924). This suggests that growth rate is not regulated within any phase, and may change, but the final proportions of a variable are genetically predetermined. From this study, since no distinct phases were seen to occur, the latter hypothesis would be favoured, with growth changing throughout the entire life history.

Sexual dimorphism of the chelae is probably a result of

selection pressure towards those animals which are most successful in sexual and aggressive encounters. The chelae are important for both of these (e.g. Bovbjerg, 1955; Stein, 1975; Ingle and Thomas, 1974). Dimorphism also occurs for the abdomen widths of males and females. Isometric growth occurs for immature females and males for this variable, but whilst mature males still exhibit this growth pattern, females show allometric growth. This is related to the requirement for a wide abdomen to carry the eggs (see also Rhodes and Holdich, 1981). the greater telson length exhibited by females may also be for protection of the eggs, since brooding animals tend to curl their abdomens and telson under them (N.B. The wider telson of males compared to females with telsons of the same length does not imply a wider telson for animals of equivalent carapace length). Thus the observation of sexual dimorphism for the total length may partly be explained by the greater length of the female telson, and partly due to a longer abdomen. Such differences are likely to be related to the requirement for carrying eggs.

Allometric growth also occurred for the weight of the animals. No sexual dimorphism was seen to occur for this variable for immature animals of either population or for mature animals from Markfield Quarry. This is because weight gains due to the greater growth of the male chelae are balanced by the greater growth of the female abdomen. Sexual dimorphism did occur for mature animals from the Leen, indicating that the chelae add appreciably more weight in this population than Markfield. Indeed, although Markfield animals have longer chelae, those of the Leen are wider, and it is in the lower, wide part which consistutes the bulk

of the chelae.

The rostrum was seen to change its dimensions isometrically, and without exception there was no sexual dimorphism. This is of note since the rostrum of *A. pallipes* is particularly distinctive (see Gordon, 1963; Thomas, 1974) and is used to separate out the species of *Austropotamobius* (Gledhill, *et. al*, 1976). Population differences of this variable did (surprisingly therefore) occur. The rostrum of the Leen animals was shorter and wider than that of the Markfield animals, whilst the Markfield animals were found to be intermediate.

Other population differences were inconsistent, but bear consideration regarding the meat yield of each population. The chelae and abdomens are the meat containing areas of crayfish, and the yields from similar sized specimens of *A. pallipes* and certain exotic species compares favourably (Rhodes, 1980). Should commercial cropping of populations occur then the males are to be favoured due to their large chelae. The extra width of the female abdomen does not in fact increase the meat yield (Lindqvist and Louekari, 1975) so the changes occurring are simply structural. Comparing the populations it may be seen that although the Leen animals have shorter chelae than those of Nanpantan or Markfield, they are wider for the same length and therefore will yield more meat. Consequently this population would be favoured commercially.

The differences between the populations may be due to environmental differences to some extent. Huner and Romaire (1978) found that *P. clarkii* from the most favourable conditions would have attained the largest size. Skvortsov (1980) considered *A. leptodactylus* from different habitats and measured similar

dimensions to this author. Several differences were seen to exist between the populations, the most obvious of which was the length of the terminal spicule of the rostrum. It was suggested that these differences were due to geographical variability, and that since morphological differences are adaptive, then the population variations reflect the response of the organism to the environment. The population showing the least variability therefore enjoys the most favourable conditions. In this study the population differences were not consistent and do not suggest that any one population was in a more favourable environment than the others. It must also be considered that some degree of genetic variability between the populations could account for the differences observed.

TABLES 5.9 - 5.16, NOTE

CARL	=	Carapace length
CARW	=	Carapace width
TOTL	=	Total length
ROSL	=	Rostrum length
ROSW	=	Rostrum width
TELL	=	Telson length
TELW	=	Telson width
ASG2	=	Width of second abdominal segment
CHEL	=	Chela length
CHEW	=	Chela width
CHIL	=	Length of moveable digit of chela
WEIGHT	=	Weight

TABLE 5.9 THE BEST-FIT REGRESSION ANALYSES FOR VARIOUS DIMENSIONS OF THE CRAYFISH *A. PALLIPES* FROM THE RIVER LEEN

VARIABLE (Y)	REFERENCE DIMENSION(x)	EQUATION	S.E.b.	r	F	P	N	GROWTH PATTERN
1. MALES								
CHEL	CARL	LOG.Y=1.2739LOG.x-0.5544	0.0108	0.9800	13922.7	<0.0001	575	POSITIVE ALLOMETRY
ASG2	CARL	Y=0.4934x-0.5466	0.0062	0.9890	6296.2	<0.001	141	ISOMETRIC
TOTL	CARL	Y=2.0450x+1.4327	0.0077	0.9953	71306.0	<0.001	677	ISOMETRIC
CARW	CARL	Y=0.5638x-1.3998	0.0031	0.9896	15828.9	<0.001	678	ISOMETRIC
TELL	CARL	Y=0.3157x+0.2798	0.0046	0.9853	4671.4	<0.001	141	ISOMETRIC
ROSL	CARL	Y=0.2720x+1.2270	0.0036	0.9883	5755.3	<0.001	139	ISOMETRIC
WEIGHT	CARL	LOG.Y=3.2942LOG.x-3.9466	0.0166	0.9926	39391.6	<0.001	594	POSITIVE ALLOMETRY
CHEW	CHEL	Y=0.4704x-0.3155	0.0035	0.9923	17611.5	<0.001	276	ISOMETRIC
CHIL	CHEL	Y=0.5508x+0.1502	0.0037	0.9939	22392.5	<0.001	276	ISOMETRIC
TELW	TELL	Y=0.7538x+0.4487	0.0098	0.9883	5901.7	<0.001	142	ISOMETRIC
ROSW	ROSL	Y=0.7681x-0.2143	0.0131	0.9806	3446.1	<0.001	139	ISOMETRIC
2. FEMALES								
CHEL	CARL	LOG.Y=1.1252LOG.x-0.3811	0.0104	0.9752	11647.9	<0.0001	602	POSITIVE ALLOMETRY
ASG2	CARL	LOG.Y=1.2141LOG.x-0.5881	0.0135	0.9898	8041.6	<0.0001	169	POSITIVE ALLOMETRY
TOTL	CARL	Y=2.1291x+0.4366	0.0096	0.9932	48749.0	<0.001	669	ISOMETRIC
CARW	CARL	Y=0.5561x-1.1110	0.0030	0.9905	34746.9	<0.001	669	ISOMETRIC
TELL	CARL	Y=0.3487x-0.2765	0.0042	0.9880	6900.2	<0.001	109	ISOMETRIC
ROSL	CARL	Y=0.2750x+1.2410	0.0029	0.9907	8824.3	<0.001	168	ISOMETRIC
WEIGHT	CARL	LOG.Y=3.1868LOG.x-3.8007	0.0217	0.9854	21629.5	<0.001	650	POSITIVE ALLOMETRY
CHEW	CHEL	Y=0.4460x+0.0893	0.0102	0.9377	1915.1	<0.001	264	ISOMETRIC
CHIL	CHEL	Y=0.5179x+0.7059	0.0116	0.9399	1992.1	<0.001	264	ISOMETRIC
TELW	TELL	Y=0.7342x+0.5781	0.0087	0.9883	7107.8	<0.001	171	ISOMETRIC
ROSW	ROSL	Y=0.9735x-0.2397	0.0104	0.9853	5549.2	<0.001	168	ISOMETRIC

TABLE 5.10 THE BEST-FIT REGRESSION ANALYSES FOR VARIOUS DIMENSIONS OF THE CRAYFISH *A. PALLIPES*

FROM MARKFIELD QUARRY

VARIABLE (Y)	REFERENCE DIMENSION(x)	EQUATION	S.E.b.	r	F	P	N	GROWTH PATTERN
1. MALES								
CHEL	CARL	LOG.Y=1.5451LOG.x-0.9744	0.0323	0.9481	2292.9	<0.0001	260	POSITIVE ALLOMETRY
ASG2	CARL	Y=0.3875x+1.8627	0.0179	0.8732	469.5	<0.001	147	ISOMETRIC
TOTL	CARL	Y=1.8522x+5.9201	0.0146	0.9920	16102.2	<0.001	262	ISOMETRIC
CARW	CARL	Y=0.5637x-2.2816	0.0081	0.9742	4865.0	<0.001	262	ISOMETRIC
TELL	CARL	Y=0.2891x+1.1296	0.0071	0.9592	1680.6	<0.001	147	ISOMETRIC
ROSL	CARL	Y=0.2838x+1.2523	0.0069	0.9559	1694.0	<0.001	161	ISOMETRIC
WEIGHT	CARL	LOG.Y=3.2190x-3.9109	0.0454	0.9792	5023.9	<0.001	218	POSITIVE ALLOMETRY
CHEW	CHEL	Y=0.3996x-0.0529	0.0108	0.9434	1369.2	<0.001	170	ISOMETRIC
CHIL	CHEL	Y=0.5375x+0.9926	0.0141	0.9402	1456.5	<0.001	170	ISOMETRIC
TELW	TELL	Y=0.7065x+0.7060	0.0176	0.9576	1609.5	<0.001	147	ISOMETRIC
ROSW	ROSL	Y=0.6743x+0.3858	0.0240	0.9115	787.3	<0.001	161	ISOMETRIC
2. FEMALES								
CHEL	CARL	LOG.Y=1.1233LOG.x-0.4061	0.0215	0.9676	2718.9	<0.0001	187	POSITIVE ALLOMETRY
ASG2	CARL	LOG.Y=1.1489LOG.x-0.5208	0.0269	0.9739	1824.2	<0.001	101	POSITIVE ALLOMETRY
TOTL	CARL	Y=2.0141x+2.7434	0.0205	0.9905	9659.8	<0.001	188	ISOMETRIC
CARW	CARL	Y=0.5641x-2.0191	0.0155	0.9374	1354.7	<0.001	188	ISOMETRIC
TELL	CARL	Y=0.3403x-0.0769	0.0082	0.9720	1711.1	<0.001	101	ISOMETRIC
ROSL	CARL	Y=0.2944x+0.9442	0.0074	0.9695	1580.6	<0.001	102	ISOMETRIC
WEIGHT	CARL	LOG.Y=3.0223LOG.x-3.6340	0.0426	0.9870	5043.3	<0.001	136	POSITIVE ALLOMETRY
CHEW	CHEL	Y=0.4150x-0.3097	0.0106	0.9637	1525.6	<0.001	118	ISOMETRIC
CHIL	CHEL	Y=0.6262x-0.6422	0.0117	0.9799	2879.1	<0.001	118	ISOMETRIC
TELW	TELL	Y=0.6980x+0.8627	0.0179	0.9681	1506.6	<0.001	102	ISOMETRIC
ROSW	ROSL	Y=0.7196x-0.1231	0.0270	0.9355	708.7	<0.001	102	ISOMETRIC

TABLE 5.11 THE BEST-FIT REGRESSION ANALYSES FOR VARIOUS DIMENSIONS OF THE CRAYFISH *A. PALLIPES* FROM NANPANTAN RESERVOIR

VARIABLE (Y)	REFERENCE DIMENSION(x)	EQUATION	S.E.b.	r	F	P	N	GROWTH PATTERN
1.MALES								
CHEL	CARL	LOG.Y=1.5620LOG.x-0.9990	0.0181	0.9753	7474.4	<0.001	386	POSITIVE ALLOMETRY
ASG2	CARL	Y=0.4723x-0.2626	0.0128	0.9901	1370.1	<0.001	28	ISOMETRIC
TOTL	CARL	Y=1.9705x+2.4910	0.0113	0.9936	30170.8	<0.001	389	ISOMETRIC
CARW	CARL	Y=0.6157x-2.7114	0.0043	0.9906	20325.6	<0.001	389	ISOMETRIC
TELL	CARL	Y=0.3027x+0.1051	0.0080	0.9908	1445.2	<0.001	28	ISOMETRIC
ROSL	CARL	Y=0.2733x+1.2285	0.0074	0.9903	1378.3	<0.001	28	ISOMETRIC
CHEW	CHEL	Y=0.4396x-0.0402	0.0181	0.9780	591.5	<0.001	28	ISOMETRIC
CHIL	CHEL	Y=0.5564x+0.0080	0.0124	0.9933	1998.8	<0.001	28	ISOMETRIC
TELW	TELL	Y=0.7502x+0.7021	0.0230	0.9876	1069.1	<0.001	28	ISOMETRIC
ROSW	ROSL	Y=0.7427x-0.2706	0.0343	0.9723	468.0	<0.001	28	ISOMETRIC
2.FEMALES								
CHEL	CARL	LOG.Y=1.2354LOG.x-0.5593	0.0138	0.9781	8054.1	<0.001	367	POSITIVE ALLOMETRY
ASG2	CARL	LOG.Y=1.1365LOG.x-0.4686	0.0584	0.9686	378.8	<0.001	27	POSITIVE ALLOMETRY
TOTL	CARL	Y=2.1116x+0.1708	0.0128	0.9932	27026.5	<0.001	373	ISOMETRIC
CARW	CARL	Y=0.6060x-2.1657	0.0074	0.9725	6480.9	<0.001	373	ISOMETRIC
TELL	CARL	Y=0.3423x-0.4465	0.0121	0.9843	804.8	<0.001	27	ISOMETRIC
ROSL	CARL	Y=0.2759x+1.2123	0.0118	0.9771	550.2	<0.001	27	ISOMETRIC
CHEW	CHEL	Y=0.4343x+0.2033	0.0184	0.9791	560.5	<0.001	25	ISOMETRIC
CHIL	CHEL	Y=0.5679x+0.0149	0.0143	0.9928	1589.2	<0.001	25	ISOMETRIC
TELW	TELL	Y=0.6972x+1.0447	0.0248	0.9840	791.8	<0.001	27	ISOMETRIC
ROSW	ROSL	Y=0.6906x+0.2444	0.0493	0.9393	195.9	<0.001	27	ISOMETRIC

TABLE 5.12 A COMPARISON OF THE DIMENSIONS WHICH SHOW ISOMETRIC GROWTH, USING THE t-STATISTIC TO COMPARE THE SLOPES OF THE REGRESSION

(i) A COMPARISON OF MALES AND FEMALES WITHIN EACH POPULATION

VARIABLE (Y)	REFERENCE DIMENSION (x)	RIVER LEEN			MARKFIELD QUARRY			NANPANTAN RESERVOIR		
		t	p	RELATIONSHIP	t	p	RELATIONSHIP	t	p	RELATIONSHIP
TOTL	CARL	9.67	<0.001	♀ > ♂	9.35	<0.001	♀ > ♂	11.67	<0.001	♀ > ♂
CARW	CARL	2.52	<0.025	♂ > ♀	0.034	>0.5	♀ = ♂	1.57	>0.1	♀ = ♂
TELL	CARL	7.51	<0.001	♀ > ♂	6.79	<0.001	♀ > ♂	3.88	<0.001	♀ > ♂
ROSL	CARL	0.93	>0.2	♀ = ♂	1.50	>0.1	♀ = ♂	0.27	>0.5	♀ = ♂
CHEW	CHEL	3.23	<0.005	♂ > ♀	1.44	>0.1	♀ = ♂	0.28	>0.5	♀ = ♂
CHIL	CHEL	3.86	<0.001	♂ > ♀	6.72	<0.001	♀ > ♂	0.87	>0.2	♀ = ♂
TELW	TELL	2.13	<0.05	♂ > ♀	0.48	>0.5	♀ = ♂	2.22	<0.05	♂ > ♀
ROSW	ROSL	0.47	>0.5	♀ = ♂	1.80	>0.05	♀ = ♂	1.23	>0.2	♀ = ♂

(ii) A COMPARISON OF THE POPULATIONS, WITHIN EACH SEX

VARIABLE (Y)	REFERENCE DIMENSION (x)	COMPARISON	MALES				FEMALES			
			t	p	RELATION-SHIP	OVERALL RELATION-SHIP	t	p	RELATION-SHIP	OVERALL RELATION-SHIP
TOTL	CARL	L v M	11.03	<0.001	L > M	L = N > M	0.14	>0.05	N/S	L = N > M
		L v N	1.44	>0.1	N/S		1.75	>0.05	N/S	
		M v N	9.28	<0.001	N > M		6.17	<0.001	N > M	
CARW	CARL	L v M	0.02	>0.5	N/S	N > L = M	0.26	>0.5	N/S	N > L = M
		L v M	5.25	<0.001	N > L		6.31	<0.001	N > L	
		M v N	8.50	<0.001	N > M		3.88	<0.001	N > M	
TELL	CARL	L v M	2.45	<0.025	L > M	L > M = N	1.35	>0.1	N/S	L = M = N
		L v N	0.37	>0.5	N/S		1.04	>0.2	N/S	
		M v N	1.90	>0.05	N/S		0.21	>0.5	N/S	
ROSL	CARL	L v M	4.30	<0.001	M > L	M = N > L	5.04	<0.001	M > L	M > L = N
		L v N	3.02	<0.005	M > L		1.06	>0.2	N/S	
		M v N	1.51	>0.1	N/S		2.19	<0.05	M > N	
CHEW	CHEL	L v M	9.83	<0.001	L > M	L > N > M	3.00	<0.005	L > M	L > M = N
		L v N	4.90	<0.001	L > N		1.06	>0.2	L > N	
		M v N	3.32	<0.005	N > M		1.57	>0.1	N/S	
CHIL	CHEL	L v M	1.34	>0.1	N/S	L = M = N	9.51	<0.001	M > L	M > N > L
		L v N	0.70	>0.4	N/S		5.26	<0.001	N > L	
		M v N	1.36	>0.1	N/S		4.81	<0.001	M > N	
TELW	TELL	L v M	1.32	>0.1	N/S	N > L = M	0.02	>0.5	N/S	L = M = N
		L v N	1.59	>0.1	N/S		0.04	>0.5	N/S	
		M v N	2.36	<0.025	N > M		0.04	>0.5	N/S	
ROSW	ROSL	L v M	4.52	<0.001	L > M	L = N > M	2.25	<0.025	L > M	L > N = M
		L v N	1.35	>0.1	N/S		3.21	<0.005	L > N	
		M v N	2.66	<0.025	N > M		0.88	>0.4	N/S	

TABLE 5.13 AN INVESTIGATION OF THE INFLEXION POINTS OF THE LOG. LINEAR REGRESSION DATA WHICH SHOWED ALLOMETRIC GROWTH AGAINST THE CARAPACE LENGTH (REFERENCE DIMENSION) COMPARED WITH SOME LINEAR REGRESSION DATA

SEX	VARIABLE	REGRESSION	POPULATION: INFLEXION POINTS (mm)			
			LEEN	MARKFIELD	NANPANTAN	POOLED*
MALES	CHEL	LOG. LINEAR	21	30	⩾ 36	
	WEIGHT	LOG. LINEAR	21	30	-	
MALES	CHEL	LINEAR	27.0	31.2	40.0	29.0
FEMALES	CHEL	LOG. LINEAR	28	30	⩾ 36	
	WEIGHT	LOG. LINEAR	22	30	-	
	ASG1	LOG. LINEAR	29	30	⩾ 36	
FEMALES	CHEL	LINEAR				30.7
	ASG1	LINEAR				24.0

* FROM RHODES AND HOLDICH (1979).

TABLE 5.14 THE REGRESSION ANALYSES ±25 mm (SEXUAL MATURITY) FOR THOSE VARIABLES WHICH, IN TABLES 5.9 - 5.11, WERE SEEN TO EXHIBIT POSITIVE ALLOMETRY

VARIABLE (x)	REFERENCE DIMENSION (Y)	SEX	POPULATION	± 25mm ?	EQUATION	S.E.b.	r	F	P	N	GROWTH PATTERN
CHEL	CARL	MALE	LEEN	+	LOG. y = 1.5273 LOG x -0.9237	0.0377	0.9300	1639.4	<0.001	258	ALLOMETRIC
			LEEN	-	LOG. Y = 1.1177 LOG x -0.3629	0.0150	0.9729	5566.2	<0.001	317	ALLOMETRIC
			MARKFIELD	+	LOG. Y = 1.6375 LOG x -1.1162	0.0414	0.9293	1563.3	<0.001	249	ALLOMETRIC
			MARKFIELD	-	LOG. Y = 1.2680 LOG x -0.5810	0.0741	0.9850	293.2	<0.001	11	ALLOMETRIC
			NANPANTAN	+	LOG. Y = 1.6443 LOG x -1.1254	0.0286	0.9611	3314.5	<0.001	276	ALLOMETRIC
			NANPANTAN	-	LOG. Y = 1.3613 LOG x -0.7199	0.0912	0.8207	222.8	<0.001	110	ALLOMETRIC
CHEL	CARL	FEMALE	LEEN	+	LOG. Y = 1.2855 LOG x -0.6224	0.0328	0.9164	1541.9	<0.001	296	ALLOMETRIC
			LEEN	-	LOG. Y = 1.0769 LOG x -0.3214	0.0229	0.9374	2203.8	<0.001	306	ISOMETRIC
			MARKFIELD	+	LOG. Y = 1.1786 LOG x -0.4893	0.0316	0.9451	1388.2	<0.001	168	ALLOMETRIC
			MARKFIELD	-	LOG. Y = 1.0381 LOG x -0.2867	0.0982	0.9317	111.9	<0.001	19	ISOMETRIC
			NANPANTAN	+	LOG. Y = 1.2442 LOG x -0.5724	0.0241	2670.5	2670.5	<0.001	267	ALLOMETRIC
			NANPANTAN	-	LOG. Y = 1.1646 LOG x -0.4637	0.0594	0.8925	383.8	<0.001	100	ALLOMETRIC
ASG2	CARL	MALE	LEEN	ALL	LOG. Y = 1.0684 LOG x -0.4236	0.0129	0.9899	6830.2	<0.001	141	ISOMETRIC
			MARKFIELD	ALL	LOG. Y = 0.8522 LOG x -0.1291	0.0487	0.8237	306.0	<0.001	147	ISOMETRIC
			NANPANTAN	ALL	LOG. Y = 1.0377 LOG x -0.3902	0.0288	0.9901	1298.8	<0.001	28	ISOMETRIC
ASG2	CARL	FEMALE	LEEN	+	LOG. Y = 1.2811 LOG x -0.6857	0.0469	0.9429	745.9	<0.001	95	ALLOMETRIC
			LEEN	-	LOG. Y = 1.0987 LOG x -0.4439	0.0195	0.9889	3179.2	<0.001	74	ISOMETRIC
			MARKFIELD	+	LOG. Y = 1.1951 LOG x -0.5894	0.0456	0.9445	685.8	<0.001	85	ALLOMETRIC
			MARKFIELD	-	LOG. Y = 1.0600 LOG x -0.4000	0.0748	0.9669	201.0	<0.001	16	ISOMETRIC
			NANPANTAN	+	LOG. Y = 1.0309 LOG x -0.3100	0.1254	0.8938	67.5	<0.001	19	ISOMETRIC
			NANPANTAN	-	LOG. Y = 1.2998 LOG x -0.6869	0.1137	0.9778	130.6	<0.001	8	ALLOMETRIC
WEIGHT	* CARL	MALE	LEEN	+	LOG. Y = 3.2956 LOG x -3.9493	0.0412	0.9798	6414.6	<0.001	269	ALLOMETRIC
			LEEN	-	LOG. Y = 3.3062 LOG x -3.9610	0.0354	0.9820	8711.6	<0.001	325	ALLOMETRIC
			MARKFIELD	+	LOG. Y = 3.2880 LOG x -4.0161	0.0511	0.9713	4148.3	<0.001	251	ALLOMETRIC
			MARKFIELD	-	LOG. Y = 3.0000 LOG x -3.59853	0.1742	0.9852	296.7	<0.001	11	ALLOMETRIC
WEIGHT	CARL	FEMALE	LEEN	+	LOG. Y = 2.9883 LOG x -3.5054	0.0450	0.9650	4404.5	<0.001	327	ALLOMETRIC
			LEEN	-	LOG. Y = 3.2240 LOG x -3.8475	0.0551	0.9562	3425.6	<0.001	323	ALLOMETRIC
			MARKFIELD	+	LOG. Y = 2.9984 LOG x -3.5969	0.0607	0.9674	2439.9	<0.001	169	ALLOMETRIC
			MARKFIELD	-	LOG. Y = 2.9096 LOG x -3.4754	0.1448	0.9796	403.9	<0.001	19	ALLOMETRIC

TABLE 5.15(i)-(iii) A COMPARISON OF THE DIMENSIONS WHICH SHOW ALLOMETRIC GROWTH, USING THE t-STATISTIC TO COMPARE THE SLOPES OF THE REGRESSION

5.15(i) A COMPARISON OF THE DATA ±MATURITY, WITHIN EACH SEX, AND EACH POPULATION

VARIABLE (Y)	REFERENCE DIMENSION (x)	SEX	POPULATION	t	p	RELATIONSHIP
CHEL	CARL	MALE	LEEN	14.84	<0.001	+ > -
		FEMALE	LEEN	7.40	<0.001	+ > -
		MALE	MARKFIELD	8.60	<0.001	+ > -
		FEMALE	MARKFIELD	3.31	<0.005	+ > -
		MALE	NANPANTAN	5.22	<0.001	+ > -
		FEMALE	NANPANTAN	2.15	<0.05	+ > -
ASG2	CARL	MALE	LEEN	-	-	ISOMETRIC
		FEMALE	LEEN	4.86	<0.001	+ > -
		MALE	MARKFIELD	-	-	ISOMETRIC
		FEMALE	MARKFIELD	2.66	<0.025	+ > -
		MALE	NANPANTAN	-	-	ISOMETRIC
		FEMALE	NANPANTAN	2.20	<0.05	- > +
WEIGHT	CARL	MALE	LEEN	0.1836	>0.05	N/S
		FEMALE	LEEN	6.88	<0.001	- > +
		MALE	MARKFIELD	4.81	<0.001	+ > -
		FEMALE	MARKFIELD	1.22	>0.2	N/S

5.15(ii) A COMPARISON OF MALES AND FEMALES WITHIN EACH POPULATION ±MATURITY (25 mm)

VARIABLE (Y)	REFERENCE DIMENSION(x)	±25mm, MATURITY	POPULATION	t	p	RELATIONSHIP
CHEL	CARL	+	LEEN	6.88	<0.001	♂ > ♀
		-	LEEN	2.12	<0.05	♂ > ♀
		+	MARKFIELD	12.15	<0.001	♂ > ♀
		-	MARKFIELD	2.54	<0.025	♂ > ♀
		+	NANPANTAN	15.11	<0.001	♂ > ♀
		-	NANPANTAN	2.53	<0.025	♂ > ♀
ASG2	CARL	+	LEEN	6.90	<0.001	♀ > ♂
		-	LEEN	1.96	>0.05	N/S
		+	MARKFIELD	7.20	<0.001	♀ > ♂
		-	MARKFIELD	4.03	<0.001	♀ > ♂
		+	NANPANTAN	0.09	>0.05	N/S
		-	NANPANTAN	4.71	<0.005	♀ > ♂
WEIGHT	CARL	+	LEEN	7.10	<0.001	♂ > ♀
		-	LEEN	1.78	>0.05	N/S
		+	MARKFIELD	1.20	>0.2	N/S
		-	MARKFIELD	0.24	>0.5	N/S

TABLE 5.15(iii) A COMPARISON OF THE POPULATIONS WITHIN EACH SEX, ± MATURITY (25 mm)

(a) MALES

VARIABLE (Y)	REFERENCE DIMENSION(x)	±25 mm, MATURITY	COMPARISON (POPULATIONS)	t	p	RELATIONSHIP	OVERALL RELATIONSHIP
CHEL	CARL	+	L v M	2.79	<0.01	M > L	M = N > L
			L v N	3.51	<0.001	N > L	
			M v N	0.19	>0.5	N/S	
		-	L v M	7.80	<0.001	M > L	M = N > L
			L v N	5.08	<0.001	N > L	
			M v N	1.04	>0.2	N/S	
ASG	CARL	ALL	L v M	6.01	<0.001	L > M	L = N > M
			L v N	1.87	>0.05	N/S	
			M v N	4.01	<0.001	N > M	
WEIGHT	CARL	+	L v M	0.16	>0.5	N/S	L = M
		-	L v M	6.78	<0.001	L > M	L > M

TABLE 5.15 (iii) A COMPARISON OF THE POPULATIONS WITHIN EACH SEX, ± MATURITY (25 mm)

(b) FEMALES

VARIABLE (Y)	REFERENCE DIMENSION(x)	±25 mm MATURITY	COMPARISON (POPULATIONS)	t	p	RELATIONSHIP	OVERALL RELATIONSHIP
CHEL	CARL	+	L v M	3.30	<0.001	L > M	L = N > M
			L v N	1.42	>0.1	N/S	
			M v N	2.41	<0.025	N > M	
		-	L v M	1.22	>0.2	N/S	M = L < N
			L v N	2.47	<0.025	N > L	
			M v N	1.90	>0.05	N/S	
ASG2	CARL	+	L v M	1.86	>0.05	N/S	L = M > N
			L v N	3.82	<0.001	L > N	
			M v N	2.47	<0.025	M > N	
		-	L v M	1.10	>0.2	N/S	L = M > N
			L v N	5.48	<0.005	N > L	
			M v N	2.72	<0.025	N > M	
WEIGHT	CARL	+	L v M	0.20	>0.5	N/S	L = M
		-	L v M	5.01	<0.001	L > M	L > M

TABLE 5.16 A SUMMARY OF THE RESULTS OF THE ANALYSIS OF RELATIVE GROWTH IN *A. PALLIPES*

(i) THE ALLOMETRIC VARIABLES

POPULATION	VARIABLE	REFERENCE DIMENSIONS	TYPE OF GROWTH EXHIBITED MALES			FEMALES			SEXUAL DIMORPHISM		GREATER GROWTH ♂ OR ♀ ?		POPULATION DIFFERENCES +		-	
			+	-	+ > - ?	+	-	+ > - ?	+	-	+	-	♂	♀	♂	♀
L	CHEL	CARL	A	A	+	A	I	+	YES	YES	♂	♂	M=N>L	L=N>M	M=N>L	N>L=M
M			A	A	+	A	I	+	YES	YES	♂	♂				
N			A	A	+	A	A	+	YES	YES	♂	♂				
L	ASG2	CARL	I	I	/	A	I	+	YES	NO	♀	=	L=N>M	L=M>N	L=N>M	N>L=M
M			I	I	/	A	I	+	YES	YES	♀	♀				
N			I	I	/	I	A	-	NO	YES	=	♀				
L	WEIGHT	CARL	A	A	N/S	A	A	-	YES	NO	♂	=	L = M	L = M	L > M	L > M
M			A	A	+	A	A	N/S	NO	NO	=	=				

WHERE L, M, N = Leen, Markfield, Nanpantan

+ = >25 mm C.L.

- = <25 mm C.L.

A = Allometric

I = Isometric

+ >- ? = Asks whether the level of allometry is greater ±25 mm C.L.?

N/S = No significant difference

5.16 (ii) THE VARIABLES WHICH EXHIBIT ONLY ISOMETRIC GROWTH

POPULATION	VARIABLE	REFERENCE DIMENSION	SEXUAL DIMORPHISM	GREATER GROWTH ♂ OR ♀?	POPULATION DIFFERENCES ♂	POPULATION DIFFERENCES ♀
L M N	TOTL	CARL	YES YES YES	FEMALE FEMALE FEMALE	L=N>M	L=N>M
L M N	CARW	CARL	YES NO NO	MALE EQUAL EQUAL	N>L=M	N>L=M
L M N	TELL	CARL	YES YES YES	FEMALE FEMALE FEMALE	N=L>M	L=M=N
L M N	ROSL	CARL	NO NO NO	EQUAL EQUAL EQUAL	M=N>L	M>L=N
L M N	CHEW	CHEL	YES NO NO	MALE EQUAL EQUAL	L>N>M	L>M=N
L M N	CHIL	CHEL	YES YES YES	MALE FEMALE FEMALE	L=M=N	M>N>L
L M N	TELW	TELL	YES NO YES	MALE EQUAL MALE	L=N>M	L=M=N
L M N	ROSW	ROSL	NO NO NO	EQUAL EQUAL EQUAL	L=N>M	L>N=M

FIG. 5.10

A plot of the standardized residuals (i.e. residual errors, vertical-axis) against the predicted standardized dependent variable (horizontal axis) where the predicted line was a linear regression of chelae length against carapace length for the Leen males, thus:

$Y = 0.9445 \times -6.0306$

This figure is reproduced from output generated using the SPSS package on the Nottingham University ICL 2900 computer, and each dot may represent several which actually occupy that space on the original digital readout from the computer. This is particularly true in the most densely represented area of the graph. The graph shows a poor fit of the predicted line compared to the actual line, which is shown to curve around that predicted.

Fig. 5.10

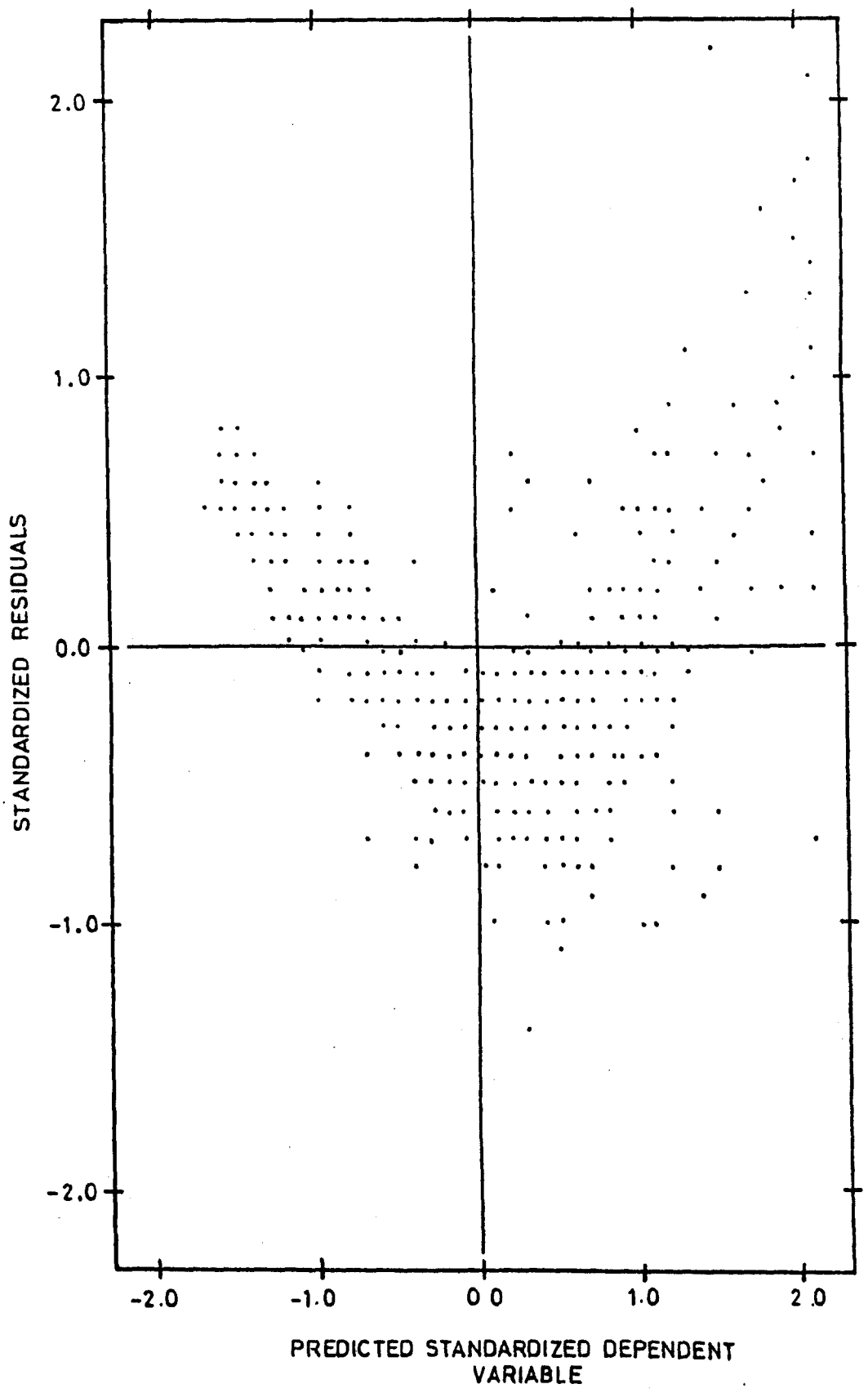

2.0
1.0
0.0
-1.0
-2.0
STANDARDIZED RESIDUALS
-2.0
-1.0
0 0
1.0
2.0
PREDICTED STANDARDIZED DEPENDENT VARIABLE

FIG. 5.11

A plot of the standardized residuals (vertical axis) against the predicted standardized dependent variable (horizontal axis) where the predicted line was a linear regression of Log. Chelae length against log. carapace length for the Leen males, thus:

LOG.Y = 1.2739 LOG.x -0.5544

The figure is reproduced from output generated using the SPSS package on the Nottingham University ICL 2900 computer, and each dot may represent several which actually occupy that space on the original digital readout from the computer. Taking the log. of each variable linearizes the true data which is shown to be a curve (Fig. 5.12) and this figure now illustrates a good fit of the predicted line with the actual data.

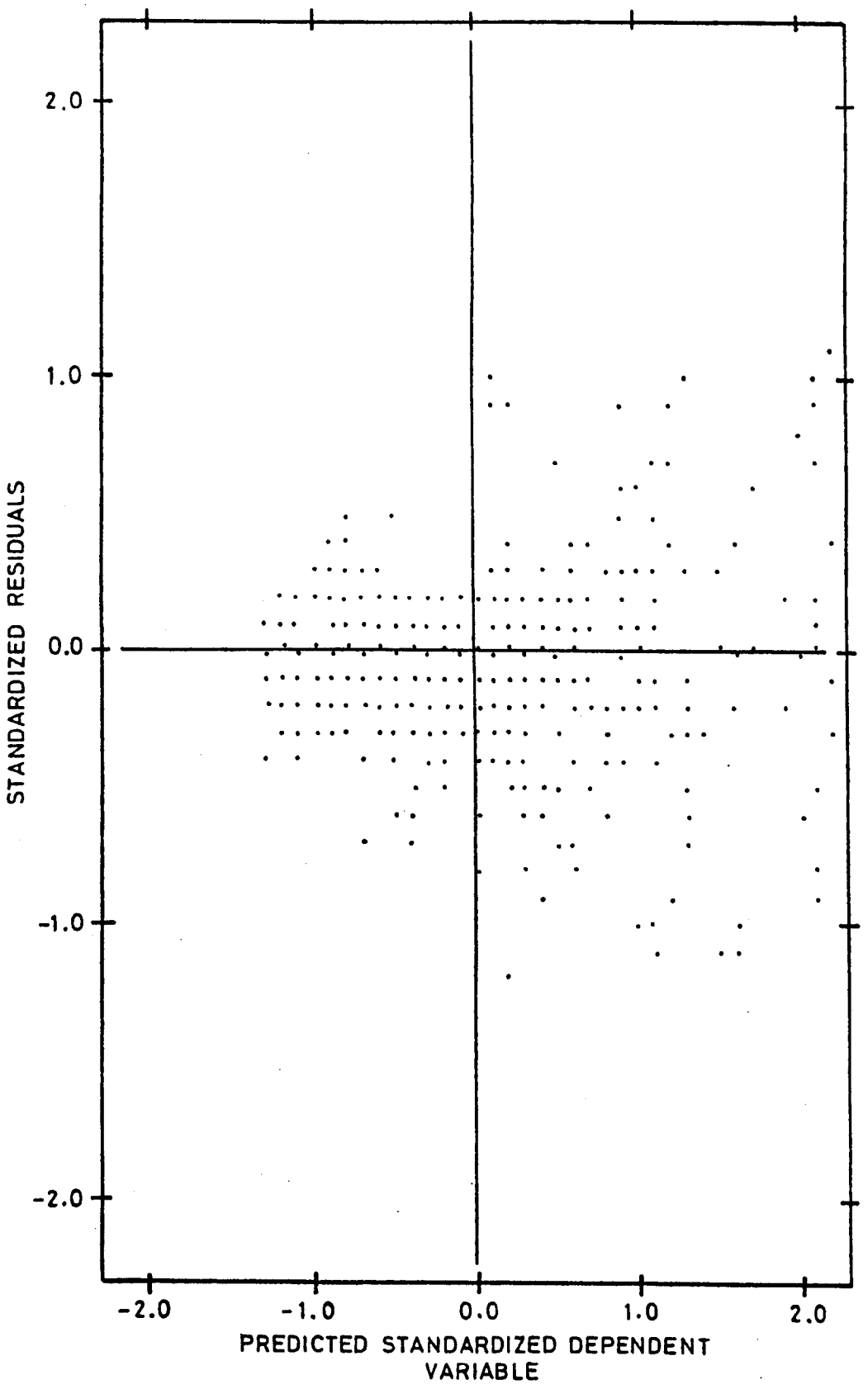
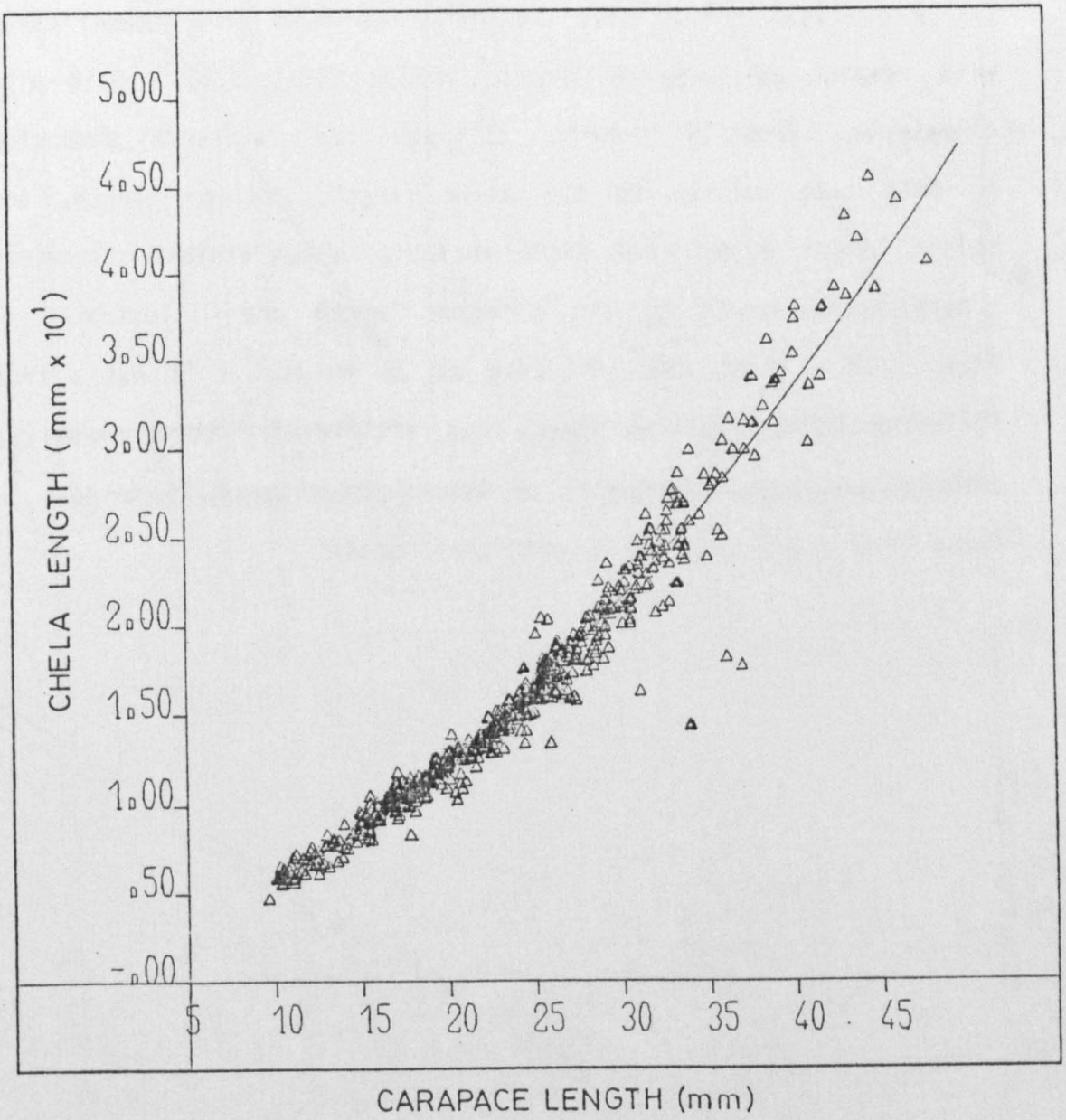

2.0
1.0
0.0
-1.0
-2.0
STANDARDIZED RESIDUALS
-2.0
-1.0
0.0
1.0
2.0
PREDICTED STANDARDIZED DEPENDENT VARIABLE

FIG. 5.12

To show the relationship between chelae length and carapace length for the males of the River Leen population.

This computer drawn figure utilizes all the relevant data collected over the two-year study period, and shows the limited scatter of data. The line fitted to the points has been drawn in by the computer as the line of best fit. It is quite clearly a curve.

FIG. 5.12 RIVER LEEN MALES, CHELA LENGTH vs CARAPACE LENGTH



FIGS. 5.13 - 5.22

These figures illustrate the growth of certain variables with respect to a reference variable, as detailed in the text (5.2). The solid line in each figure is that for the males of the population whilst the broken line relates to the females.

Figs. 5.13 - 5.15 show the variables which grow isometrically with respect to carapace length, whilst Figs. 5.16 - 5.18 also illustrate isometric growth, although the reference dimension in this case relates to the chela length, rostrum length, and telson length as detailed. Those variables which exhibit allometric growth with respect to the carapace length are illustrated in Figs. 5.19 - 5.22. The inflexion at 25 mm (C.L.) is not a true inflexion point, but one which has artificially been chosen to coincide with the known size at sexual maturity of 25 mm (C.L.). These figures are drawn on a logarithmic scale.

FIG. 5.13(i)-(iii) THE ISOMETRIC VARIABLES OF THE RIVER LEEN POPULATION

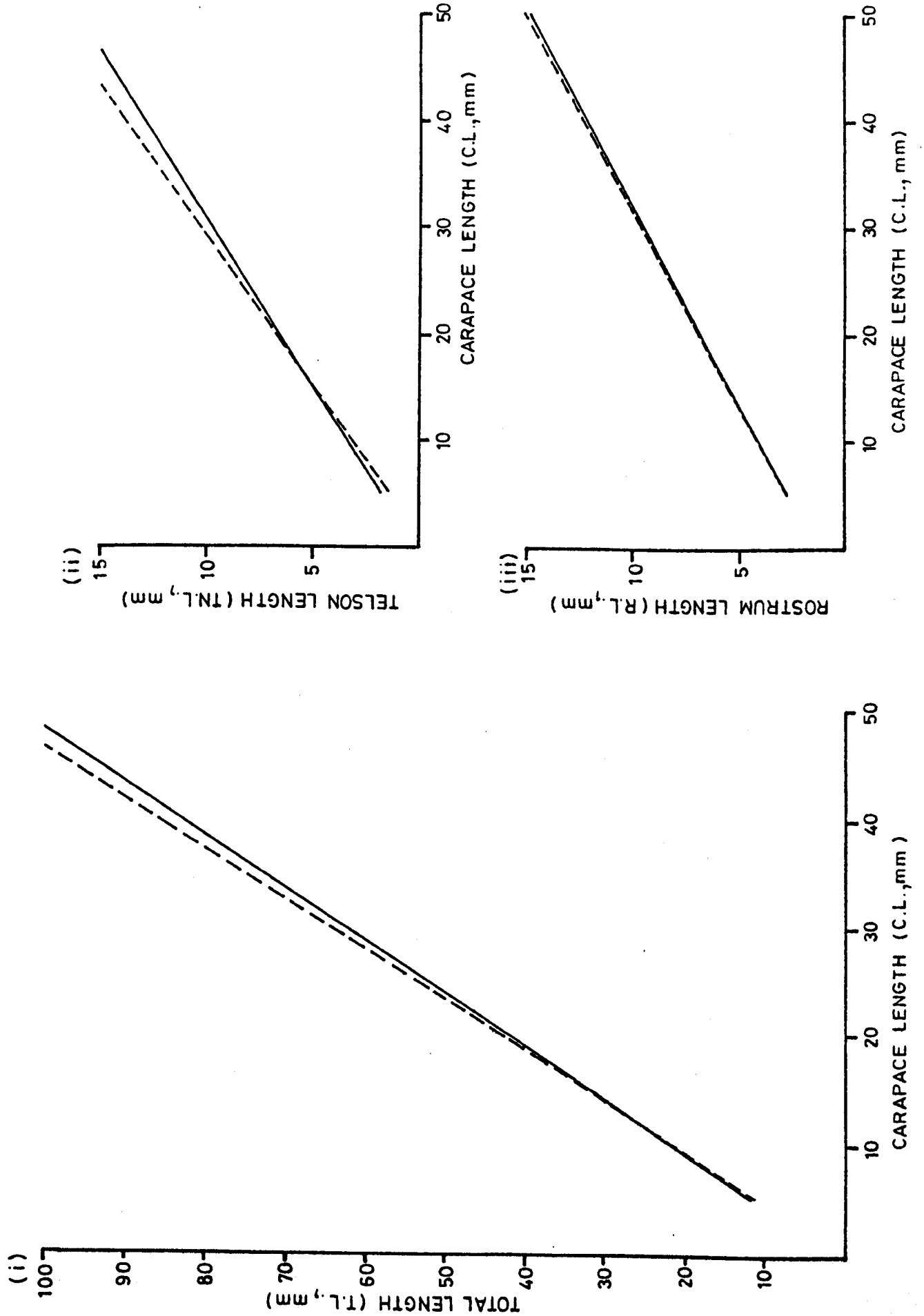


FIG. 5.14(i)-(iii) THE ISOMETRIC VARIABLES OF THE MARKFIELD QUARRY POPULATION

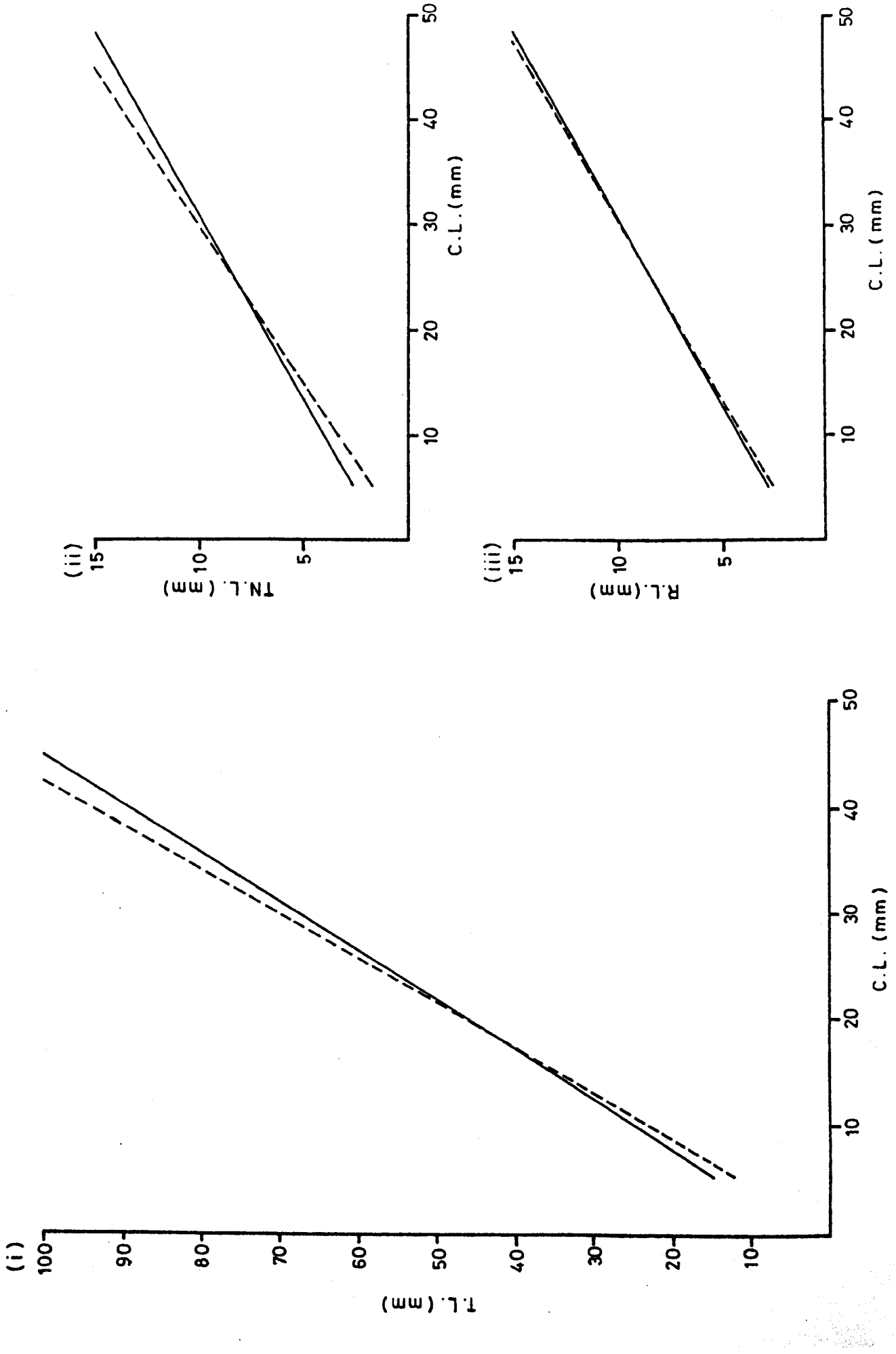


FIG. 5.15(i)-(iii) THE ISOMETRIC VARIABLES OF THE NANPANTAN POPULATION

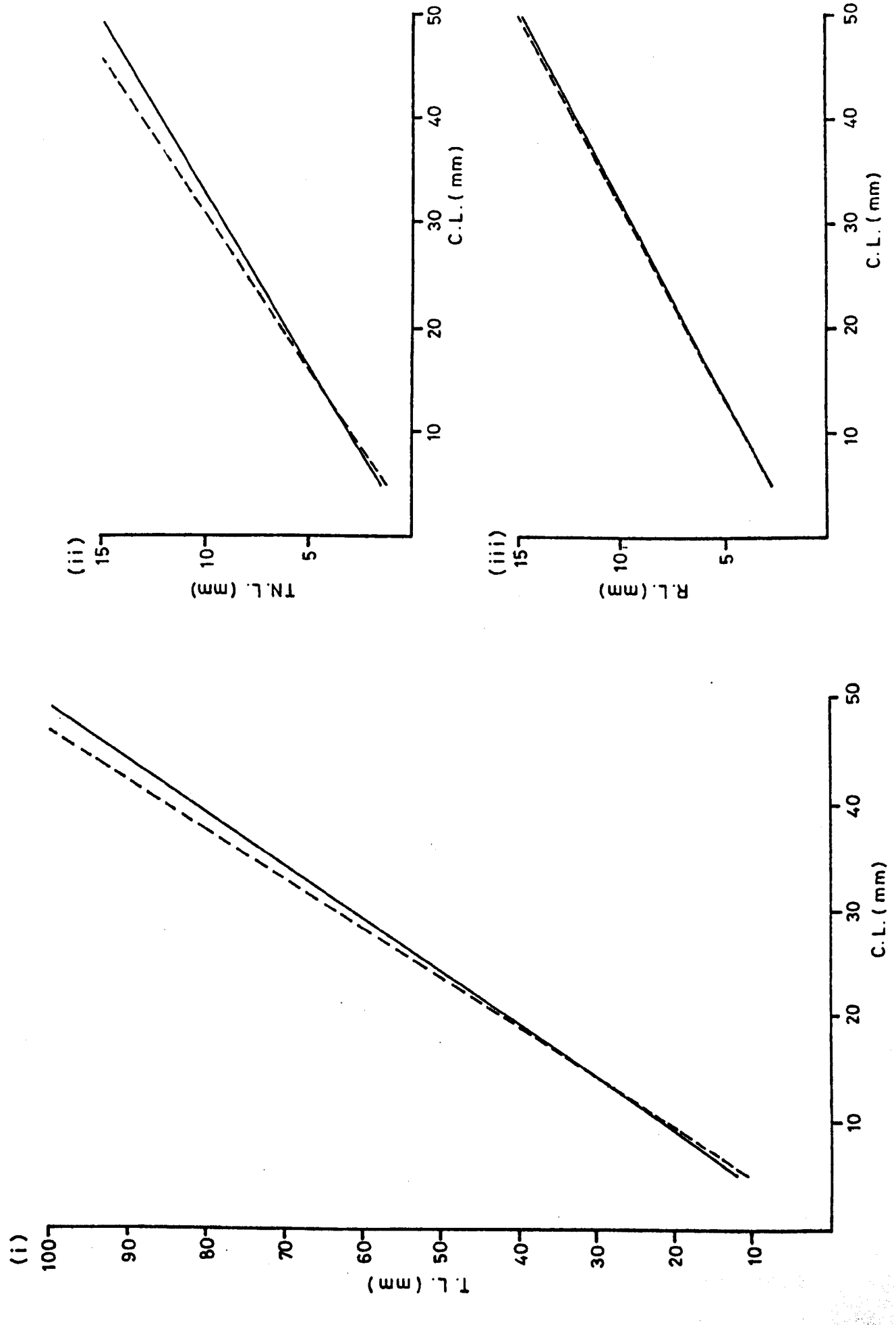


FIG. 5.16(i)-(iv) THE VARIABLES WHICH ARE ISOMETRIC TO EACH OTHER, LEEN POPULATION

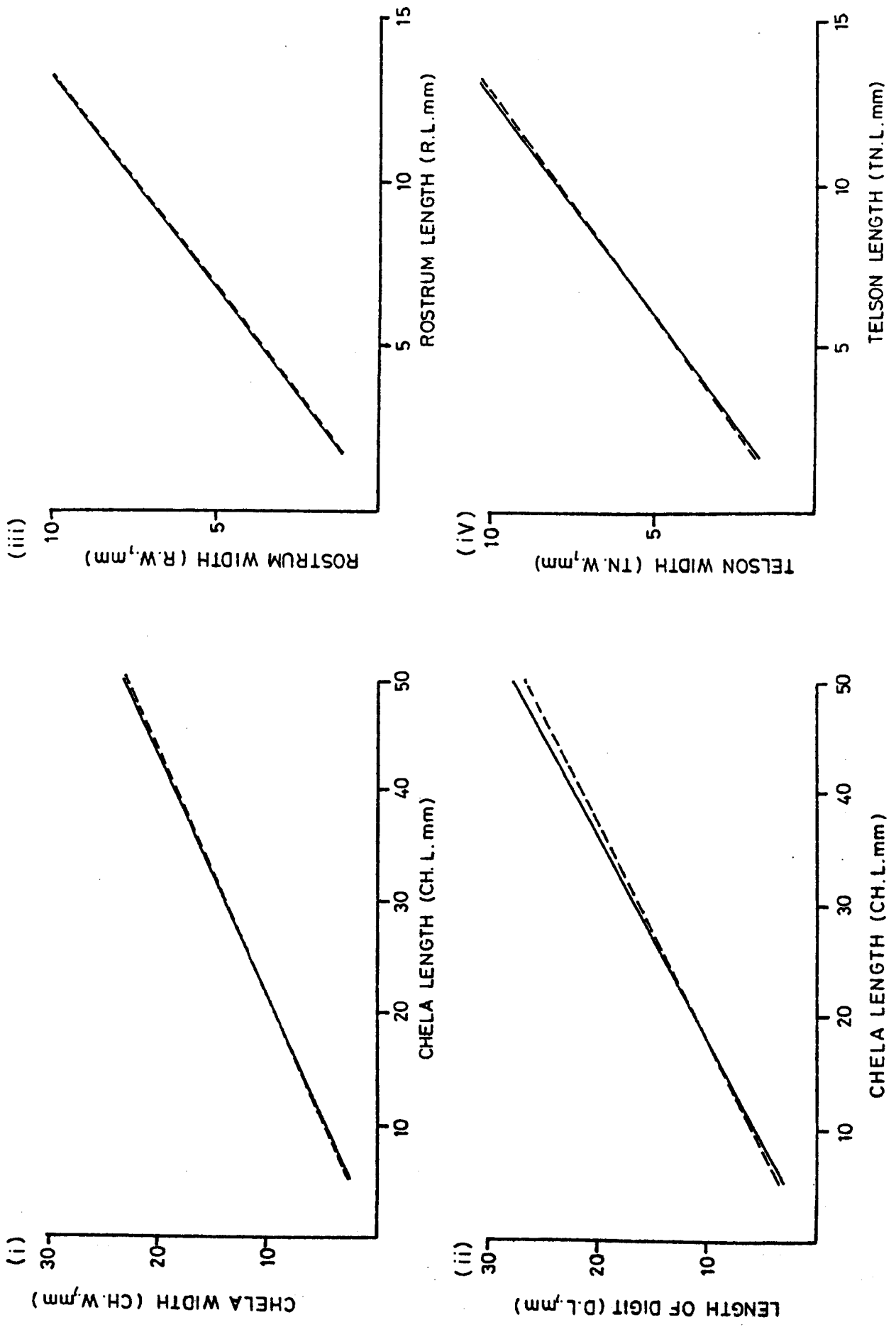


FIG. 5.17(i)-(iv) THE VARIABLES WHICH ARE ISOMETRIC TO EACH OTHER, MARKFIELD POPULATION

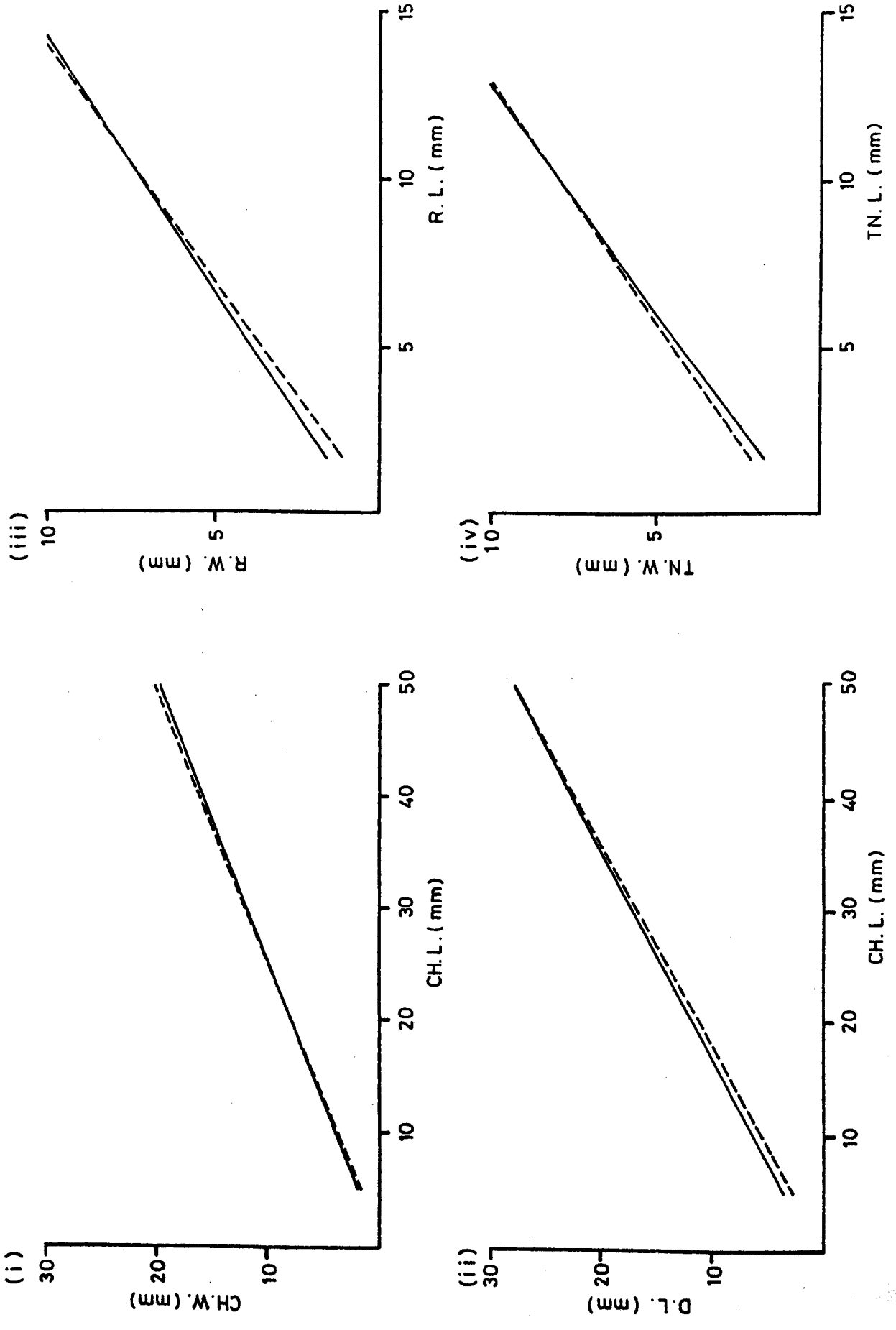


FIG. 5.18(i)-(iv) THE VARIABLES WHICH ARE ISOMETRIC TO EACH OTHER, NANPANTAN POPULATION

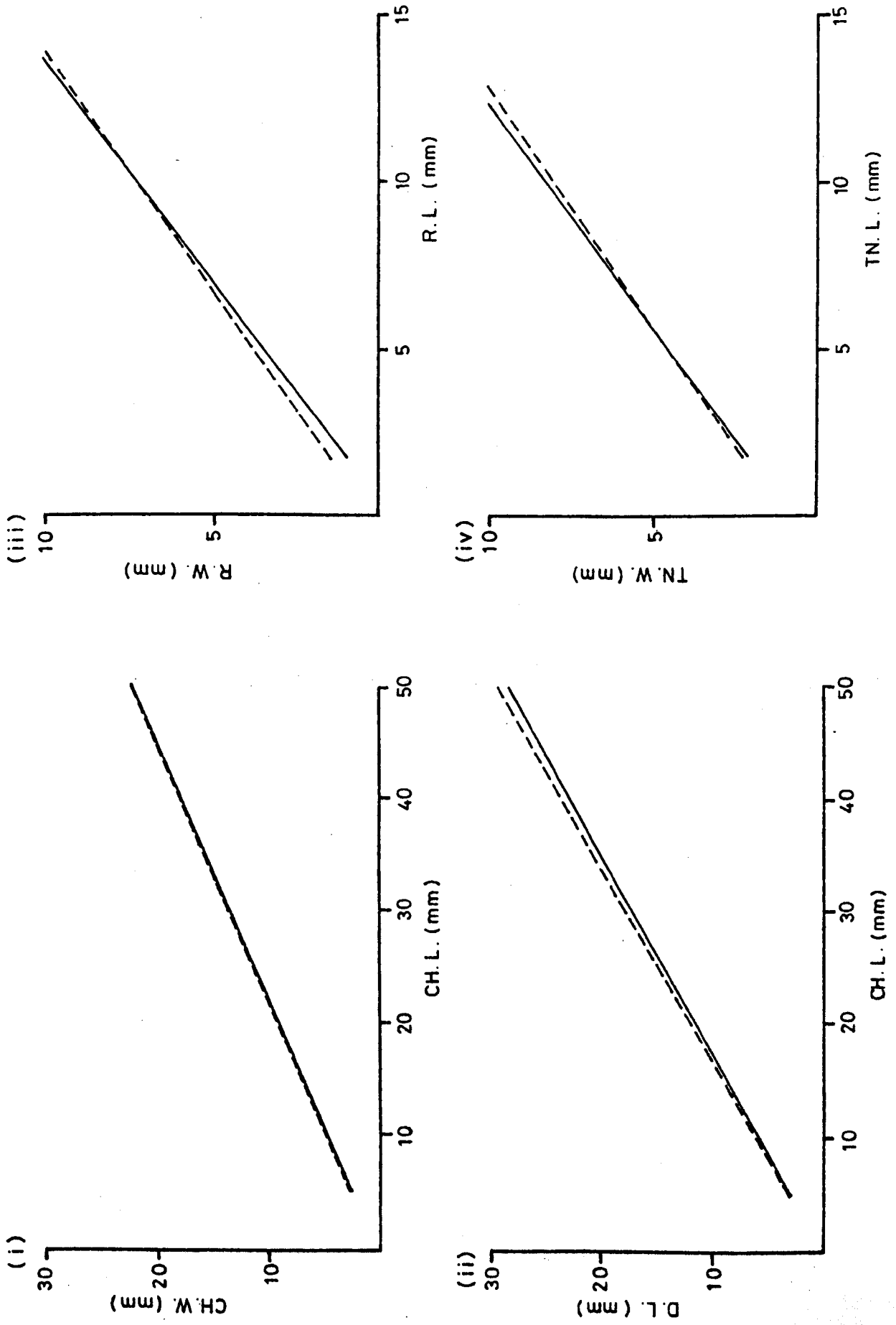
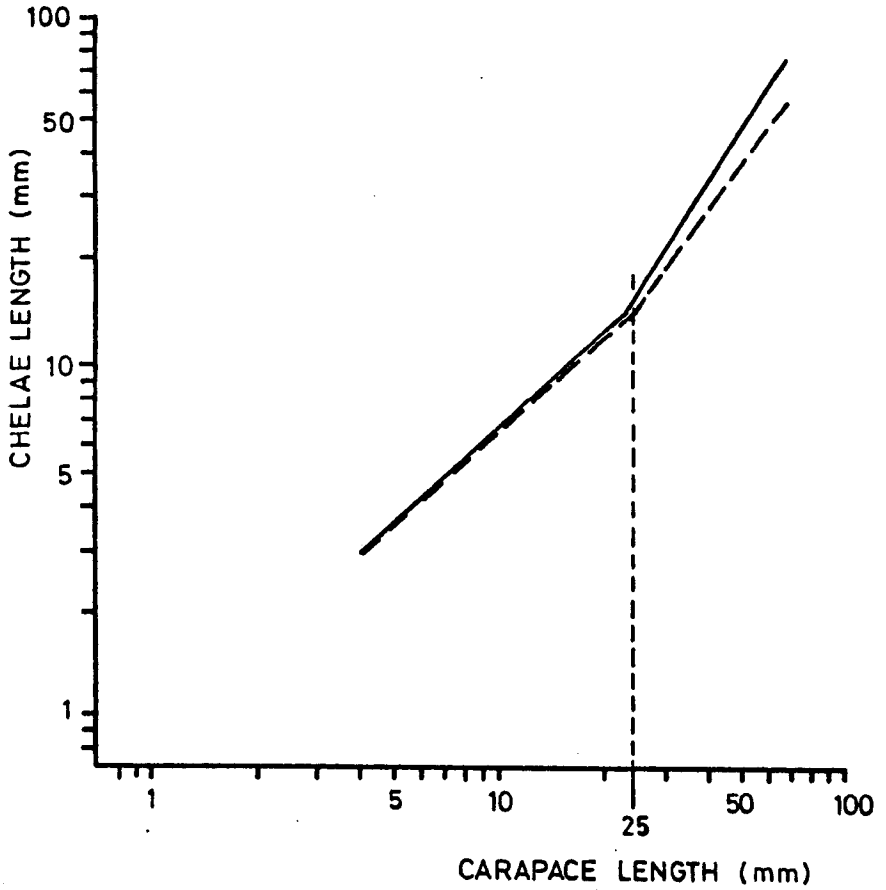


FIG. 5.19 THE ALLOMETRIC VARIABLES OF THE RIVER LEEN POPULATION



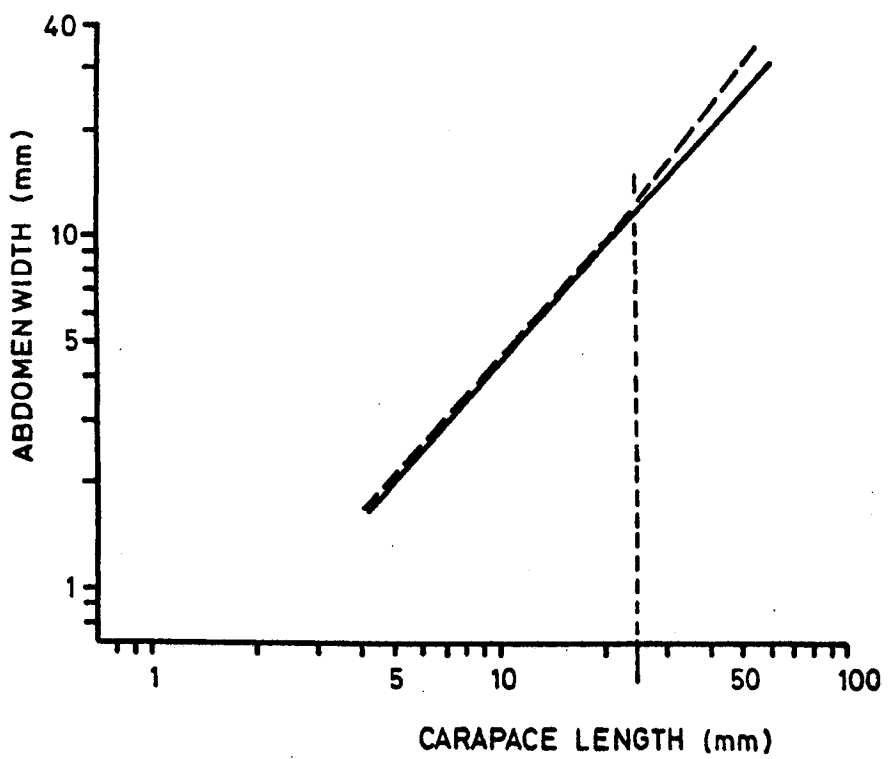
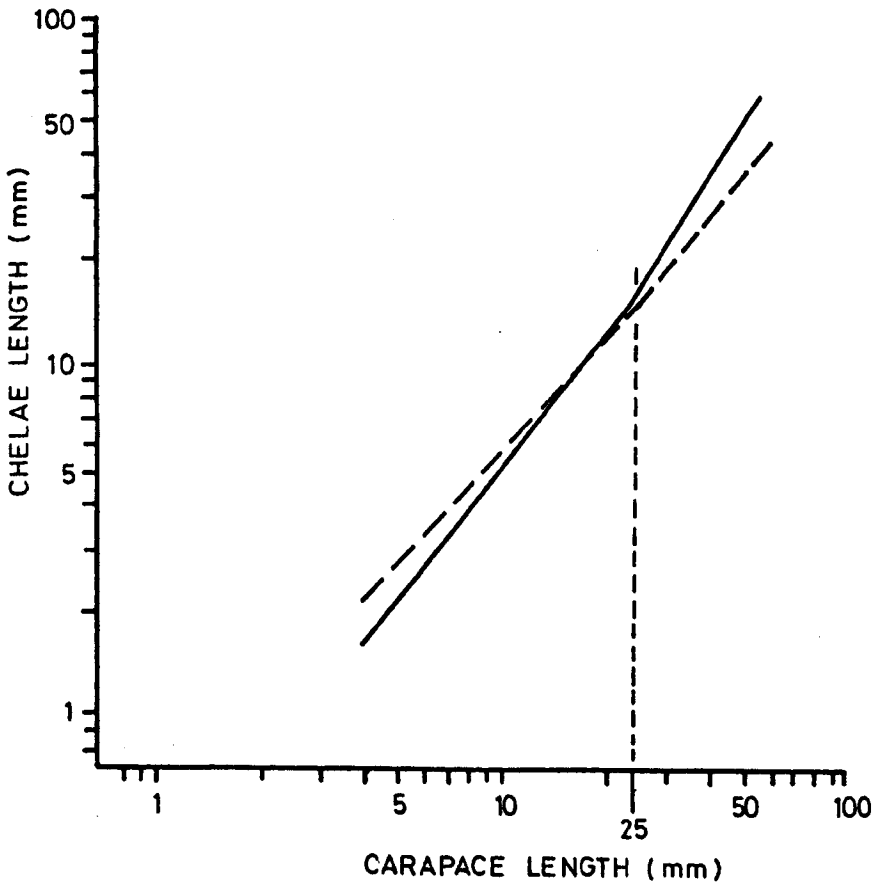


FIG. 5.20 THE ALLOMETRIC VARIABLES OF THE MARKFIELD QUARRY POPULATION



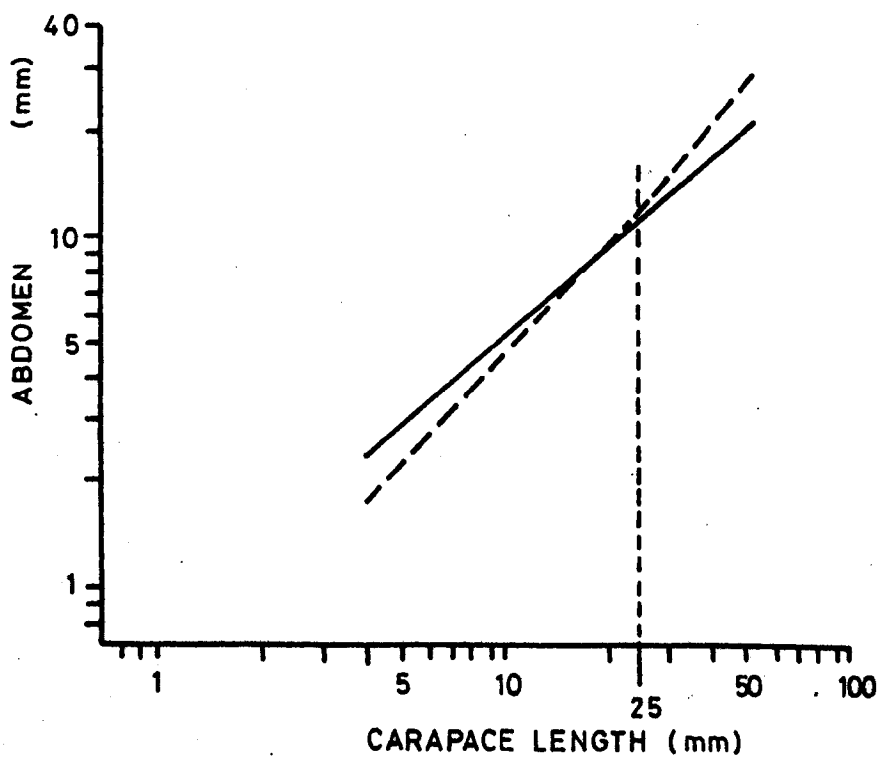
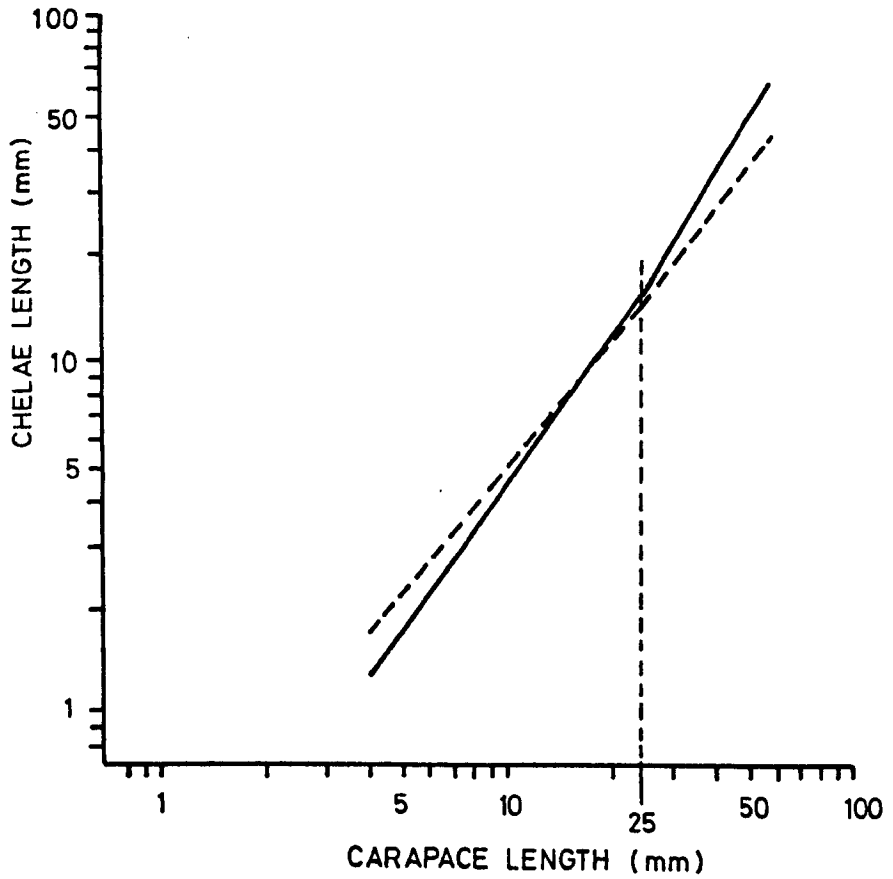


FIG. 5.21 THE ALLOMETRIC VARIABLES OF THE NANPANTAN POPULATION



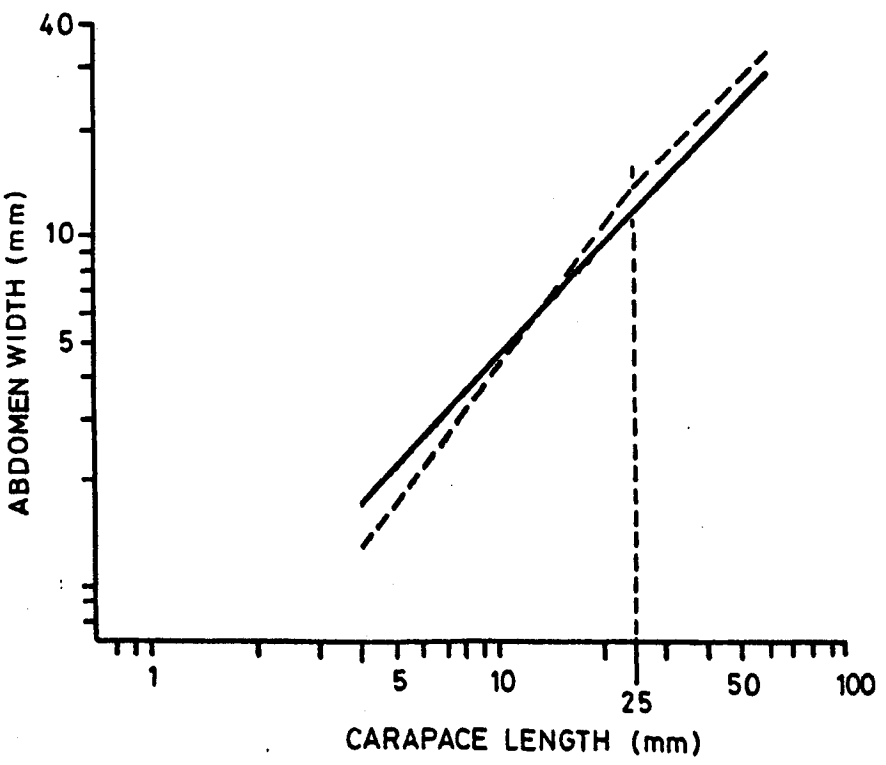
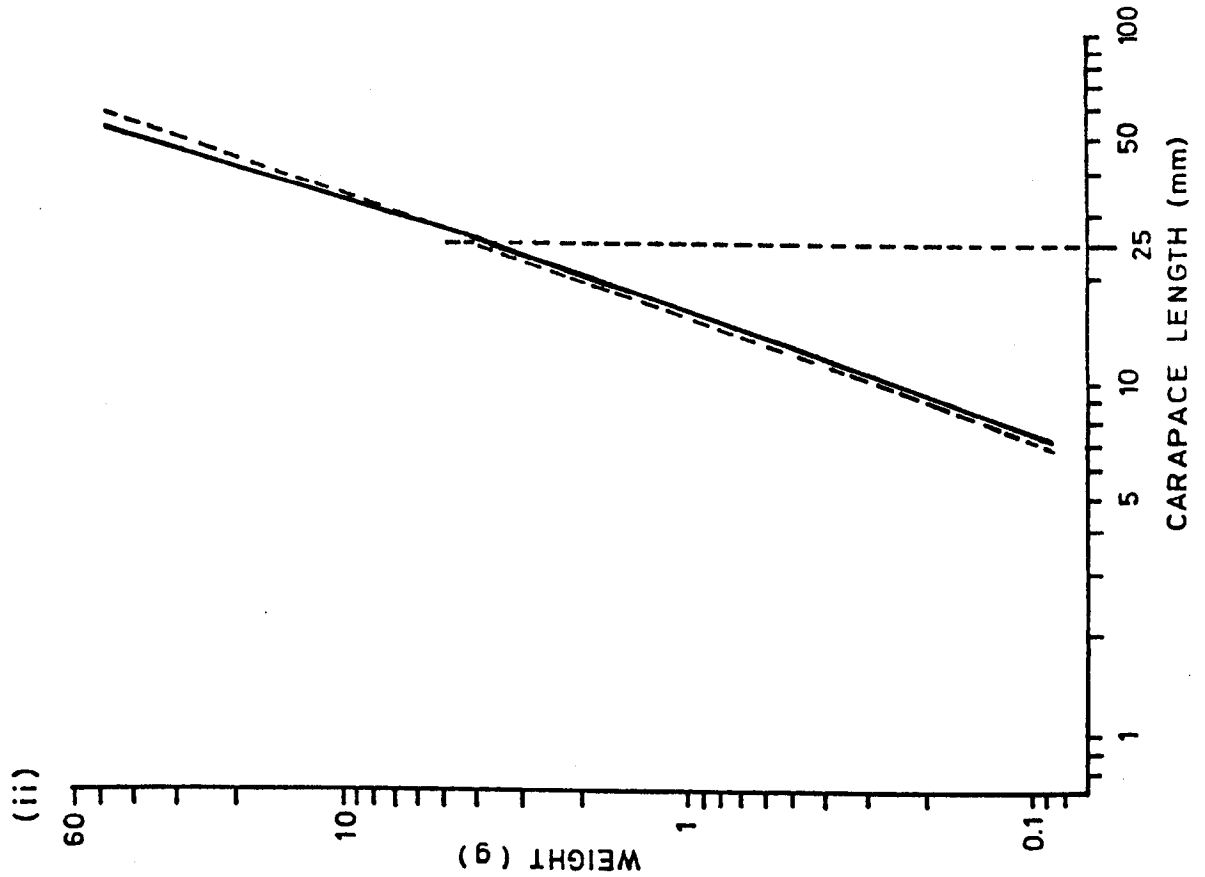
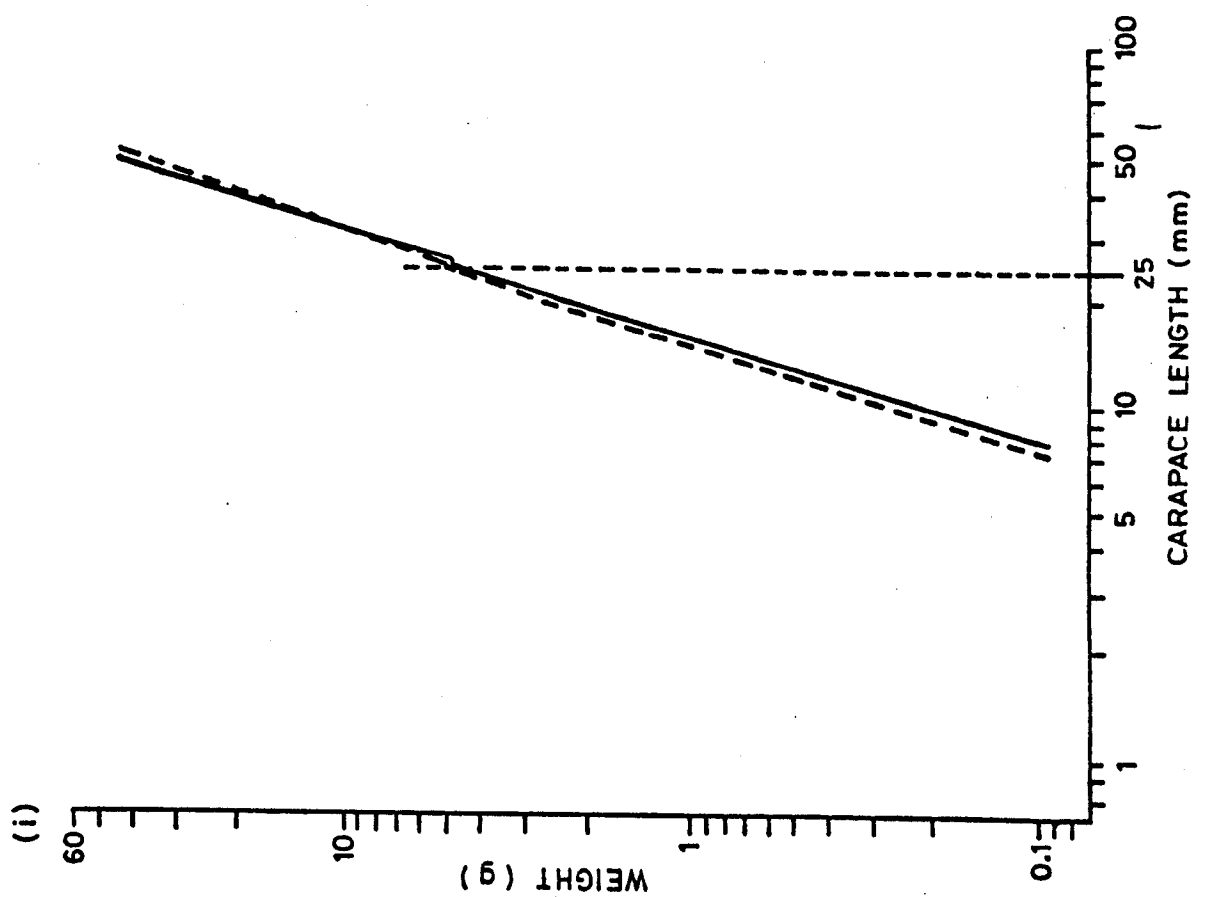


FIG. 5.22 TO SHOW THE ALLOMETRIC RELATIONSHIP OF WEIGHT AGAINST CARAPACE LENGTH FOR THE (i) LEEN, (ii) MARKFIELD POPULATIONS





GENERAL DISCUSSION TO PART I

At the beginning of Part I of this thesis it was stated that the two major concerns of British crayfish biologists today were to investigate the basic ecology of *A. pallipes* (which until recently was virtually unknown), and also to examine the feasibility of exploiting this species commercially as a food resource. The recent interest has centred on the introduction of exotic crayfish species into Britain (Part I, Introduction), particularly *Pacifastacus leniusculus*. Such introductions have been made in an endeavour to meet the demands met by renewed interest in Britain towards the gastronomic qualities of crayfish. In the past it was perhaps more common than in recent years for these crustaceans to be included on the menu, but today increasingly more restaurants are seen to offer them. In Continental Europe crayfish have long been recognized as a delicacy. Consequently when native European stocks were destroyed by the 'crayfish plague', caused by the fungus *Aphanomyces astaci*, attempts were made to introduce a viable alternative. *P. leniusculus* proved to be the favoured candidate, being more similar to *Astacus astacus*, the major commercial species previously, than were other species. It originates from North-western U.S.A. and Canada, and has superior fecundity and growth to *Astacus astacus* whilst retaining the same delicious flavour. Ironically, however, it may also harbour the 'plague', acting as a carrier for spores of *Aphanomyces astaci*. This disease is thought to be indigenous to North America. Consequently N. American crayfish species have developed an immunity to the disease, whilst European stock are 'oversusceptible' and may be erradicated by its introduction. It was first thought to have

arrived in Europe in 1860, in Italy. The major vector is probably man, and due to the lack of interest in crayfish in Britain in the past, and due to the English Channel, it was never introduced. Today, however, introductions of exotic species are occurring, and it is with the fear of the crayfish plague in mind that the recent upsurge of interest has arisen. This is particularly pertinent now that it is believed that the disease may already affect certain British crayfish populations.

Regarding the ecology of *A. pallipes* various factors are of interest - what would be the effect upon British waterways should our native species be destroyed? How might *A. pallipes* fare in competition with *P. leniusculus*? What factors naturally limit crayfish populations? May a decline in a crayfish population be associated with changing conditions of water quality?, an important point to discover if the plague is also suspected. What factors in the life history of natural populations may be controlled in order to promote the viability of *A. pallipes* as a food resource? Next, regarding the potential of *A. pallipes* as an alternative to *P. leniusculus* - How does the meat yield compare? Will a population be able to withstand exploitation and regenerate? What is a suitable marketable size? How fast does *A. pallipes* grow? What sort of population densities occur naturally which might support exploitation? What proportion of a population is of an exploitable size? What are the factors which limit population size and growth?

It may be seen that there are many questions to be answered. Some of these have been covered in this study, and others only touched on here will require further examination. Certain questions

have been answered by other authors, whilst others remain totally unexamined. For example, the reported introductions of *P. leniusculus* into rivers in the South of England known to be inhabited by *A. pallipes* must certainly form the basis of an extremely important and interesting study as to how the two species might interact in direct competition. The following discussion therefore serves both to summarize the work covered by this author and also to put it in the context of some of the questions asked above (references may be found in the relevant part of the text).

Firstly the ecological aspects of this study. A review was conducted of the trophic relations of *A. pallipes* (1.3). Crayfish fit into the food web as both predator and prey. They are also totally omnivorous and eat animal matter, macrophytic vegetation, and detritus. In view of the large populations which exist in some areas (4.1) their role must be quite substantial, and they may play a great part in preventing early ageing and eutrophication of water bodies. Should *A. pallipes* be erradicated by pollution or disease therefore, the effects may be quite substantial, and problems of weed choked rivers and lakes have even been suggested. These problems would not arise if *A. pallipes* were erradicated due to competition from an exotic species since the foreign crayfish would presumably occupy the same ecological niche. No evidence exists, however, to suggest that *A. pallipes* may not be the dominant species in competition with *P. leniusculus*, although in Europe where it has to compete with other crayfish, *A. pallipes* is confined to small streams. In Britain it occupies a much wider ecological range (3.3).

Should destruction of populations of *A. pallipes* occur, it

is far more likely that rather than direct competition, the cause will be due either to pollution, or to the disease *Aphanomyces astaci*, with the disease spreading more quickly than recolonization of the waterways by the host, *P. leniusculus*, can occur. Consequently the problem of weed choked rivers, if it is a real one, could occur, along with associated commercial losses due to the effect on leisure activities such as fishing and boating. Now that it is suspected that the crayfish plague may already be present in Britain (Part I, Introduction) it is essential to try and contain the disease. Unchecked, however, the disease may still spread only relatively slowly. The reason for this is twofold. In Europe where crayfish are caught for consumption, the disease may be transmitted on the fishing gears, or by introductions of crayfish to other water bodies by the fishermen. In Britain this practice is not so widespread, if it occurs at all, thus preventing one path of transmission. Secondly, evidence was found to suggest that *A. pallipes* may have a home range (3.3). In the absence of man, therefore, it is possible that the disease could be contained to certain populations, unless of course all populations are overlapping! Clearly this is not the case as exemplified by both of the populations studied by this author (2.1, 2.2).

The life history of *A. pallipes* is also described (3.2). It was found that the timing of events in the life history such as the onset of moulting and hatching are dependent upon water temperature. This factor was also of primary importance in determining the rate of growth experienced by different populations (5.1), and consequently the age at which sexual maturity was seen to occur differed between the populations. Size appears

to be of greater importance than age in determining maturity. The frequency of events during the life history are also seen to differ between populations, and between sexes. Moulting was more frequent at higher temperatures, and was more frequent for small rather than large crayfish. Males moulted more frequently than ovigerous females (3.2(i)). At the end of the moulting period reproduction occurs, and whether it was changing photoperiod or the reduction in ambient temperatures that induced mating was not investigated. Such factors, however, bear some future consideration since these, and others such as the temperature dependency for enhanced growth, are of potential value concerning the development of culture programmes which could be introduced for *A. pallipes*. Indeed, the effects of temperature on the growth of juvenile *A. pallipes*, and on artificially incubated eggs, have already been conducted (Rhodes, 1980).

Fecundity is another factor of importance should a culture programme be desired. One would be interested to know if it were possible to increase the number of eggs per individual. It seems that fecundity may be related to population density and food availability, and temperature may also play a part (3.2(ii)). Consequently the ideal combination of these factors must be determined prior to instigating a culture programme. The isolation of berried females is also of importance and provision should be incorporated into the design of the operation to enable isolation, even if it is only through an excess of hides. This point is apparent from field studies when distorted sex ratios occurred during the breeding season, resulting from the inactivity of berried females (4.2).

The factors governing the nationwide distribution of *A. pallipes* such as the nature of the underlying substrata, and pH of the water have been covered elsewhere (Jay and Holdich, 1981). Local distribution of crayfish within a particular water body may vary (3.3). Hide availability was found to be of primary importance in this respect. Consequently it may frequently be possible to attribute the decimation of crayfish populations to man, due to various activities such as dredging and channelization of rivers, which destroy the natural habitat and remove hides. Hide availability also appears to regulate population densities (3.3,4.1). Details were made available concerning population densities of *A. pallipes* in Markfield Quarry and the River Leen, and similar densities presumably may potentially occur in equivalent water bodies elsewhere in Britain. This is apparently the case for other localities which have been examined and compared with the Midlands population(4.1). Such information combined with that relating to the known distribution of *A. pallipes* will undoubtably help in the assessment of the impact of possible introductions of exotic species. On examination of a particular water body, if crayfish population densities prove to be less than might be expected, then factors such as whether the water quality has altered recently, or whether there is a high incidence of the disease caused by *Thelohania contejeanii* (4.2) need to be examined. Thus it will be possible to assess whether these are causative factors of the low population density before other factors such as competition from exotic species or the crayfish plague may be blamed.

Crayfish appear to be sensitive to changes in water quality

(3.3, see also Part II). Consequently, some of the relatively recent practices of man, such as the spraying of agricultural land with pesticides, and the production of industrial effluents have had an effect on the local, and probably also, large scale distribution of *A. pallipes*. Pollution will have been responsible for the decline, and even erradication, of crayfish from some water bodies. It is apparent from the density dependent nature of the fecundity of a population (3.2(ii)), however, that should sudden accidental short term pollution occur, for example, causing a dramatic decrease in population numbers, then compensation is posssible to regulate the population size. Today the trend is to clean up polluted waterways and attempt to restore them as viable fisheries (3.3). The evidence for a home range, however, might indicate that recolonization of such systems by *A. pallipes* may be a slow process. Migration out of the home range may not occur unless overpopulation resulted. Since population size is controlled by density dependent feedback mechanisms such as the regulation of fecundity (3.2(ii)), such a situation may not be common. Abrahamsson (1972a) has also reported that recolonization of Swedish waters by *Astacus astacus* proceeded only slowly. Thus to reintroduce crayfish populations into restored water bodies more quickly, assistance by man may be required. Kossakowski (1971) has suggested that this may most successfully be achieved by the introduction of sexually mature animals just prior to the breeding season. These would be taken from a reservoir of stock animals and more females than males could be introduced since a single male may copulate with several females. Markfield Quarry would prove to be an excellent choice for such a reservoir of crayfish stock.

This population is devoid of crayfish diseased with *T.contejeanii* (4.2). Hence one of the problems of artificial introductions is overcome, that of the spread of disease should stock accidentally be added to a water body which already contains crayfish. If the restocking programme was being conducted with future commercial cropping in mind, however, then the Markfield stock may not be the best choice due to the narrow chelae which are one of the major meat containing areas (5.2). The seeding of areas with commercially cultured juveniles, it is suggested, is not feasible due to the high cost of production of seed and high juvenile mortalities which occur (Kossakowski, 1971).

The growth of *A. pallipes* in the Midlands has also been examined (5.1, 5.2). This is potentially useful for answering the question, 'What factors in the life history of *A. pallipes* may be controlled to promote its viability as a food resource?' These are related to the frequency of moulting discussed above, since each moult represents a successive growth increment. Moulting, and thus growth, was seen to be temperature dependent. However, it is also apparent that the actual increment achieved at moulting may itself be affected by various factors (5.1). Amongst these are population density, food availability, hide availability, damage and disease. Consequently any culture programme should need to consider these in addition to temperature and photoperiod. The relative growth, also examined (5.2) is important for assessing meat yields from *A. pallipes* and by comparison with exotic species the yields from *A. pallipes* compare favourably, so on these grounds alone it would form a viable alternative species. However, this study also found that different populations of *A. pallipes* exhibited

differences in the relative growth of certain variables. For example, crayfish from Markfield Quarry had significantly more narrow chelae than those of equivalent size from the River Leen. Such differences may be due to environmental conditions, in which case it would be to the advantage of the biologist investigating the culture of *A. pallipes* to determine what factors lead to animals with broader chelae. Alternatively these differences may be genetically based, in which case selective breeding would be required. Indeed, a study of *A. pallipes* from different geographical regions, using gel-electrophoresis to separate the proteins may prove to be a very interesting project. It may throw light on the question of whether geographically isolated populations are indeed genetically different, or whether differences observed in growth rate, size at sexual maturity, allometry, and fecundity are entirely dependent upon environmental conditions.

Regarding the potential of *A. pallipes* as an alternative food source to the introduction of exotic species, from a purely ecological point of view it would be highly desirable to exploit the native potential. The introduction of alien species can sometimes have disastrous effects and should not be encouraged. However, from a commercial point of view *A. pallipes* would not appear to be a feasible alternative, and in Europe where local species have traditionally been consumed it was not a favoured species and is said to be economically unimportant.

If *A. pallipes* is to be exploited then two alternatives are available, to crop natural populations, or to culture the species. The generally accepted market size for crayfish is 90-100 mm total length which represents approximately 8 or 9 years growing

time for *A. pallipes* (5.1). Even applying ideal conditions based on the suggestions made above it is unlikely that the growth period could be reduced to a sufficiently short time to make it economically justified. Consequently the latter alternative may be discounted on these grounds, quite apart from the problems of providing sufficient space to prevent cannibalism and aggressive interaction resulting in the loss of chelae, the meat containing areas (see 4.21).

It has been seen that reasonably large natural populations of *A. pallipes* occur throughout Britain (4.1) and thus potential may exist for cropping natural populations. In rivers such as the Leen the population size is relatively small and would not justify exploitation on a commercial scale, or would soon become overexploited. The larger populations such as Markfield Quarry could probably withstand some exploitation, and the reduced competition may even have a beneficial effect resulting in increased growth rates of those animals remaining. In general the capacity of a population to regenerate through increased fecundity (3.2(ii)) would mean that some exploitation could be carried by the stock. However, these arguments are based on consideration of the population size alone. The actual proportion of animals within a population which are at a marketable size is so small (4.2) that cropping of natural populations would not seem feasible. Fishing could not be sustained and would have to be restricted to a very short season due to the rapidity with which market sized crayfish could be removed, and the slow growth and thus long time period in order to replace them. It has been suggested that the seeding of natural populations could be conducted in order to increase

the numbers of animals reaching a marketable size. However, the density dependent feedback mechanisms which appear to operate within a population would result in a reduction in fecundity (3.2(ii)) and through competition would lead to reduced growth rates (5.1). The increased competition for food resources and hides would thus mean that high juvenile mortalities would occur, and a large population of small animals would result, defeating the object of the seeding exercise.

In order to overcome some of the problems discussed and enable exploitation of the native stocks it may be advantageous to lower the acceptable market size. This would mean that a higher proportion of the population could be cropped, competition would thus be reduced, and seeding may now be feasible. Sexual maturity is achieved after 2 - 3 years at about 50 mm total length and so it would be possible to crop all animals of greater than 50 mm. However, the egg numbers carried increases with increasing size (3.2(ii)) and so this policy may have disastrous effects on the fecundity of a population. Furthermore market-acceptability may be a problem since crayfish are only approximately 20 - 30% meat and the smaller animals would have very small chelae containing little meat (5.2).

In conclusion therefore it would appear that *A. pallipes* does not form a commercially viable alternative to the faster growing exotic species such as *P. leniusculus*. Exploitation of the stocks however, could be feasible, and even beneficial preventing stunted populations, although only on an artisanal domestic scale, and not as a cash crop. What is envisaged is something along the lines of the Scandinavian countries where at specified times

of the year crayfish of certain sizes may be caught by local communities. This has resulted in 'crayfish-parties' and the crustacean is regarded as a delicacy rather than a staple food.

PART II

THE EFFECTS OF CERTAIN POLLUTANTS ON *A. PALLIPES*

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CHAPTER 1

INTRODUCTION

Crayfish are now absent from many rivers they used to inhabit in the region of the Severn-Trent Water Authority (S.T.W.A.) (Roberts, pers. comm.). Both heavy metal (Davies, 1964) and pesticide (Bowler, 1979) pollution have been implicated with regard to *Austropotamobius pallipes*, but to date the only studies of the effects of pollution on this crayfish species have concerned the effects of abnormal pH values (Jay and Holdich, 1976). Consequently it was decided to examine the effects of two potential pollutants, one heavy metal and one pesticide, and as the results relate to *A. pallipes* they form a unique study. (One other study has examined normal zinc metabolism in *A. pallipes*, but did not treat it as a pollutant (Bryan, 1967)). Further justification for studying the effects of pollutants upon *A. pallipes* derives from the arguments presented in Part I relating to the potential of exploiting the native British crayfish as a food resource. If it is to be consumed, the dangers of bioaccumulation of certain toxic substances, and the possibility of their transmission to man must be established. Furthermore, with the restoration of rivers in the Severn-Trent region, and the possibility of restocking them with *A. pallipes*, as discussed in Part I, tests on the lethal and sub-lethal effects of specific pollutants will enable assessment as to the feasibility of such restocking programmes.

Pollution occurs when the levels of any element or compound in the environment exceed a certain critical value and cease

to be either beneficial or neutral in their effect to an organism, and become harmful. Any single element or compound can therefore only be termed a pollutant when its concentration in the immediate environment makes it toxic to an organism. The potential pollutants, or toxicants, that it was decided to study were cadmium, as the heavy metal, and Lindane as the pesticide. Cadmium was chosen after consideration of the potential sources of heavy metal pollution in the specific area of the River Leen, and rivers in the area of the S.T.W.A. Cadmium may be found in phosphate rock fertilizers at levels of 1.8 p.p.m., and in superphosphate fertilizers at levels up to 8.9 p.p.m. (Shroeder *et.al.*, quoted in Club *et.al.*1975). Since the upper reaches of the Leen are in agricultural land, run-off from the land may constitute a source of cadmium pollution in the aquatic environment. Textile mill effluents contain up to 0.06g of cadmium per cubic metre (Walsh,*et.al.*1980), and certainly in the past this would have represented a considerable source of cadmium pollution into the River Leen (see Part I, 1.1(i)). In mining areas concentrations of up to $10\,\mu g l^{-1}$ may be found in aquatic environments (Piotrowski and Coleman, 1980), and there are coal mines along the West Branch of the Leen, and also after the confluence of the two branches (see Part I, 1.1(i)). In addition, consideration of data provided by the S.T.W.A. and summarized in Table 1.1 shows that although cadmium does not represent the heavy metal with the highest concentrations, it does occur consistently, and in measurable quantities in the Severn-Trent area. Thus cadmium was chosen. (The result obtained by the author of 0.28 p.p.m. cadmium in the River Leen after the confluence of the two branches seems particularly high, and

possibly some contamination may have occurred. That cadmium was detected, and also increased concentrations of other metals by comparison with the East Branch of the Leen were detected, however, does indicate a source of heavy metal pollution in the West Branch of the Leen, presumably from mining activities. Unfortunately this reading was not taken again and checked).

The sources of potential pesticide pollution are from agricultural run-off, which indeed constitutes the major single source of gradual pollution (Edwards, 1977). Since the upper reaches of the Leen are subject to agricultural run-off it seems feasible that pesticides could be found in the river. However, no data was available for the Leen, but S.T.W.A. data is summarized in Table 1.2 for other rivers in the Severn-Trent region. Of all the insecticides screened for, Lindane proved to occur the most consistently on a monthly basis, and also it occurred in the highest concentrations. It is an organochlorine compound which is slow to degrade remaining in an active form for a considerable period of time. It was hence the obvious candidate for study as a potential pesticide pollutant.

Cadmium is described as a heavy metal, but this term includes all metals of atomic weight greater than that of sodium, and having a specific gravity in excess of 5 (Piotrowski and Coleman, 1980). Thus it includes some 70 metals, only a few of which may be described as being of broad environmental concern. Metals are an integral part of both the environment and living matter, and some are 'essential-elements'. Others have no evident positive biological influence and their only known effects are harmful. It is these metals which are of concern. Mercury, lead and antimony

tend to be the most toxic, whilst metals of intermediate toxicity are copper, cadmium, vanadium, cobalt and nickel. The least toxic are tin, chromium, molybdenum and managanese.

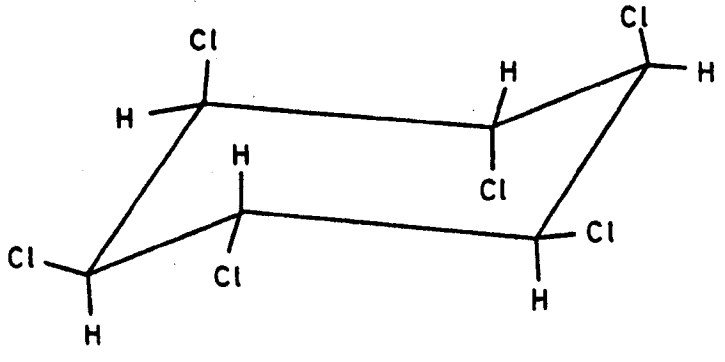
Cadmium is related to zinc, and in nature they occur together in a ratio of 1:100 to 1:1000. Naturally occurring levels in the environment are 0.01-0.7 $\mu g g^{-1}$ in agricultural soils, less than 1 $ng l^{-1}$ in fresh water, and 0.1-20 $ng m^{-3}$ in the atmosphere. Cadmium may also occur in food from an unpolluted source, varying from 0.05-0.1 $\mu g g^{-1}$ in wheat to 0.5-1.0 $\mu g g^{-1}$ in shellfish and crabs (Piotrowski and Coleman, 1980). It is obtained as a biproduct of zinc smelting, and is used in pigments, electroplating, batteries, and plastic stabilizers. In addition to the sources mentioned above, as a pollutant it occurs in areas where zinc is mined and produced. It enters the atmosphere from refuse inceration and the disposal of cadmium containing products, and also from the wear of car tyres which contain cadmium as an impurity used in the zinc oxide in them. The deposition rate of cadmium from the atmosphere varies between 40-400 $\mu g m^{-3}$ per month in urban areas, and it will enter the soil and waterways where it may be taken up by plants and micro-organisms.

Pollution of the aquatic environment additionally occurs from pulp mill effluents (Rathore *et. al.*, 1979) and in sludge from combined industrial and domestic sewage works. In contaminated waters dissolved cadmium concentrations are dependent upon pH. In conditions of neutral and alkaline pH high concentrations of cadmium may be found on suspended particles, and levels in sediments may be greater than 100 $\mu g g^{-1}$ (Piotrowski and Coleman, 1980). The ability of malacostracan decapods of economic importance

to man (for review see: Mees, 1983) to accumulate heavy metals from the environment in their tissues (see 4.1) is of potential concern. The toxic effects of cadmium have been well documented in humans, and include kidney damage and obstructive lung disease. In Japan the disease Itai-Itai has also been attributed to cadmium poisoning, resulting from the consumption of rice grown on cadmium polluted soil.

Lindane (gamma-1,2,3,4,5,6 Benzene-hexachloride (or, Hexachlorocyclohexane)) does not occur naturally in the environment. It is a man-made compound developed specifically for its ability to kill insect pests, and is used as an insecticide in crop protection, and also as a therapeutic agent in human and veterinary science (Herbst and Bodenstein, 1972). Benzene-hexachloride is a broad spectrum organochlorine insecticide made by the direct action of chlorine on benzene in ultra-violet light (Demozay and Marechal, 1972a). Technical grade BHC is a mixture of 5 configurational isomers, but it is the gamma isomer which has the insecticidal properties (Demozay and Marechal, 1972a; US EPA-440, 1980). Lindane is the trade name for the gamma isomer which is concentrated from about 10-18% in Technical grade BHC to 99-100% purity as Lindane. The insecticidal properties of Technical grade BHC and Lindane are thus quite different.

The structural formula of Lindane is as follows;



It is stable to light, heat, air, carbon dioxide and strong acids, but is unstable with alkalis, forming trichlorobenzene and hydrogen chloride (Demozay and Marechal, 1972b). Thus it is relatively stable in the environment and can retain its insecticidal properties for some time. Compared with other organochlorine pesticides it is relatively soluble in water, although at high concentrations necessary for its application, solubility may be a problem, and so Lindane may be obtained in the form of wettable powders and dusts, and emulsifiable concentrates.

In the environment Lindane has a shorter existence in soil than other organochlorine compounds such as D.D.T., Dieldrin, or Endrin (Sieper, 1972). After application the concentration of active ingredients is reduced first by physical and chemical means (due to high volatility and co-distillation with water vapour) and then by biochemical breakdown which proceeds preferentially under anaerobic conditions. The precise agency for the breakdown of Lindane to its alpha and delta isomers is not known, and there is no evidence for the existence of a λBHC dehydrochlorinase equivalent to p.p.' -D.D.T. dehydrochlorinase, an enzyme known to act anaerobically to breakdown D.D.T. to D.D.E. (Newland *et. al.*, 1969). Nevertheless degradation of Lindane by soil organisms does occur and at the rate of about 10% in 6 weeks (US EPA-440,1980).

Pollution of aquatic environments by Lindane may occur when the pesticide, bound to soil particles, is desorbed by rain water and enters lakes and rivers as agricultural run-off. Contamination may also occur by liquid wastes from manufacturing plants, and by fall-out from the atmosphere. The higher water solubility of Lindane compared with other chlorinated hydrocarbons leads

to relatively higher residues in the aquatic environment, and it is very stable in both fresh and sea water. However, it still disappears relatively quickly due to the action of secondary mechanisms such as co-distillation with evaporating water, adsorption onto sediments, and biological degradation (Sieper, 1972). Other ways it may enter aquatic environments are from use against mosquito larvae (Bodenstein, 1972), and from contamination of rivers by sheep dips containing Lindane (Hynes, 1961). This latter route of potential pollution, however, may cease to exist since resistance to λBHC by ticks on cattle and sheep is now worldwide (Tucker, 1982). The United States Environmental Protection Agency have defined 'safe' levels of Lindane in the aquatic environment, and state that the maximum concentration should not exceed 2 $\mu g l^{-1}$ and the 24 hour average should only be 0.08$\mu g l^{-1}$ in fresh waters (US EPA-440, 1980). The maximum levels for the Severn-Trent area shown in Table 1.2 are below this value. The ability of decapod crustaceans including *A. pallipes* to accumulate Lindane from the aquatic environment into their tissues (4.2) could be of potential concern. Levels which may result in increased risk of cancer in man are estimated to be of the order of 10^{-5} to 10^{-7} (weight:weight) and recommended criteria are that concentrations should not exceed 0.92 to 92 $ng l^{-1}$ in drinking water supplies (US EPA-440, 1980). However, in tests on mammals Lindane has been found to produce no teratogeneic or mutagenic effects and acceptable intake levels for humans proposed by FAO/WHO which produce no effects, are high, at 0.0125 mg Kg^{-1} body weight, with a safety factor of 100 (Herbst and Bodenstein, 1972).

Section II then, aims to examine the effects of both cadmium

and Lindane upon *A. pallipes*. Their toxicity is examined (3) and the possibility of bioaccumulation of toxicant within the crayfish tissues is studied (4.1, 4.2). The metabolic response of *A. pallipes* to each toxicant is examined by consideration of their effects upon the respiration and oxygen consumption of excised gill tissue (5), and finally a transmission electron microscope (T.E.M.) study of the chief sites of uptake of toxicant, the gills and hepatopancreas, was conducted. The results of this study, however, failed to show any cellular damage as has been demonstrated for other custacean species for both heavy metals (e.g. Marine shrimp - *Paratya tasmaniensis* (Lake and Thorpe, 1974); Marine shrimp - *Panaeus duorarum* (Couch, 1977); freshwater prawns - *Machrobranchium* and *Caradina* (Ghate and Mulherkar, 1979); freshwater cladocercan- *Daphnia magna* (Griffiths, 1980)), and pesticide like substances (marine shrimp - *Penaeus duorarum* and polychlorinated biphenyls (Couch *et. al.*, 1974); estuarine crab - *Rhithropanopeus harrissii* and insect growth regulators (Christiansen and Costlow, 1982)). Lindane has also been shown to cause cellular changes in insects (Hassanein, quoted in Demozay and Marchal, 1972c). Since no ultrastructural changes were detected in *A. pallipes* after exposure even to high concentrations of either toxicant, and also to low concentrations for long periods of time, this part of the study has not been dealt with separately, and reference is simply made in the text to this study when it is relevant to the discussion.

TABLE 1.1 THE MAXIMUM CONCENTRATIONS OF CERTAIN HEAVY METALS FOUND IN RIVERS WITHIN THE JURISDICTION OF THE SEVERN-TRENT WATER AUTHORITY

Concentrations are given as mgl^{-1}, and 1 mgl^{-1} is equivalent to 1 p.p.m. They relate to the period 01-01-78 to 01-01-79, being the year prior to commencement of this thesis, and data was obtained on request as a computer print-out from the S.T.W.A. Data relating to the levels of heavy metals in the River Leen was only given for two sampling sites (corresponding to sites 7 and 8 in Part I Fig. 1.1), and consequently data obtained by the author (see 4.1) is also given for heavy metal concentrations found in water at the River Leen population study area, and immediately after the confluence of the East and West branches of the River. This data was obtained in June 1982. Data is also presented for heavy metals found in Markfield Quarry.

RIVER SAMPLED	CONCENTRATIONS OF METAL IONS (mgl^{-1})							
	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn
TRENT (TRENT BRIDGE)	0.01	0.05	0.06	0.05	0.09	0.18	3.5	0.32
EREWASH	0.02	0.05	0.05	0.09	0.08	0.16	3.0	0.47
SOAR	<0.01	0.04	0.06	0.07	0.12	0.35	4.5	0.98
DERWENT	0.02	0.09	0.06	0.02	0.29	0.26	7.5	0.39
LEEN, TRENT CONFLUENCE	0.01	0.02	0.04	0.01	0.20	0.14	-	-
LEEN, LENTON	0.01	0.01	0.02	<0.01	<0.01	0.02	-	-
LEEN, STUDY AREA	<0.01	-	0.06	<0.01	<0.01	0.01	0.30	0.06
LEEN, E & W CONFLUENCE	0.28	-	0.37	0.05	0.20	0.76	2.20	0.54
MARKFIELD QUARRY	<0.01	-	0.01	<0.01	<0.01	0.01	0.20	0.04

TABLE 1.2 THE MAXIMUM CONCENTRATIONS OF CERTAIN PESTICIDES FOUND IN RIVERS WITHIN THE JURISDICTION OF THE SEVERN-TRENT WATER AUTHORITY

The data was obtained from the same source as Table 1.1 and relates to the same time period. Results are expressed in $\mu g l^{-1}$, and $1 \mu g l^{-1}$ is equivalent to 0.001 p.p.m. No data was available for the River Leen or Markfield Quarry. Several other pesticides were screened for, but did not occur in detectable quantities.

RIVER SAMPLED	CONCENTRATION OF PESTICIDES ($\mu g l^{-1}$)					
	LINDANE (λHCH)	DIEL-DRIN	ENDRIN	ALDRIN	HEPTA-CHLOR EPOXIPE	p.p. D.D.T.
TRENT (TRENT BRIDGE)	0.10	0.20	0.35	-	-	-
EREWASH	0.52	0.18	-	-	0.02	0.06
SOAR	0.16	0.04	-	0.02	-	-
DERWENT	0.20	0.11	-	0.01	0.01	-

CHAPTER 2

GENERAL MATERIALS AND METHODS EMPLOYED IN PART II

The crayfish used for studies of the effects of pollution on *Austropotamobius pallipes* were collected from two sites, Markfield Quarry, and Nanpantan Reservoir, in the method described in Part I (2.1(iii), 2.1(iv)). Captured animals were quickly brought to the University in insulated containers and then maintained in large concrete holding tanks until required. The two populations were not mixed. The floor of each tank was covered with gravel and broken bricks and drainpipes which acted as hides, and crumbled classroom chalk was also added to the tanks in order to help maintain calcium concentrations in the water. The water supply was Nottingham mains water, and was constantly running, being fed into the tanks via a sprinkler head in order to increase aeration of the water. The tanks were situated outside and thus were subject to natural seasonal variations in ambient conditions. During the holding period the crayfish were fed regularly with commercial trout pellets supplemented occasionally by swan mussels and ox-liver. In addition a natural flora and fauna developed in the tanks which consisted of *Cladophora* , diving beetles, and amphipods such as *Crangonyx pseudogracilis*, which may also have supplemented the diet.

Animals required for experimental work were removed from the holding tank, were sexed and measured, and then placed in divided plastic tanks with vigorous aeration, and under the appropriate conditions of light and temperature (see individual chapters) for a period of 72 hours. The usual conditions were 16 hours light to 8 hours of darkness, and maintained at a

temperature of 10°C in a coldroom, which was approximately the average temperature encountered under natural conditions (see Part I, Table 1.3 and Fig. 3.6a). 72 hours was the equilibration period during which time the crayfish were not fed in order to allow the gut to clear. After this period the water was changed, and toxicant was added as necessary, this being day 1 of the experiment.

Throughout the experimental period the water used was taken from a large glass aquarium (1.5 m x 0.5 m x 0.75 m), originally filled with Nottingham mains water. The aquarium contained gravel and classroom chalk, and the water was circulated through an activated charcoal filter. The tank was aerated, and water to be used in an experiment was not removed until the original mains water had been allowed to circulate in the system for one week, so that most of the chlorine present in the water could be removed. The pH of the water was 7.4-7.6, alkalinity 200 p.p.m., total hardness 190 p.p.m., nitrate 3.5 p.p.m., phosphate 4.9 p.p.m. (obtained using La Motte Chemicals water quality analysis kit), and the oxygen concentration was 10-11 p.p.m. On addition to the experimental tanks the stock tank water was further aerated using an airstone prior to addition of the toxicant. During any short term experiments aeration was not conducted in case of volatilization of the toxicant, particularly Lindane, although aeration has very little effect in the levels of cadmium in water (Collier *et. al.*, 1973).

The experimental set up employed consisted of a static system, and used plastic aquaria divided into six compartments using perspex dividers. Glass aquaria would have been preferable, but

were not available in sufficient quantities. With plastic aquaria and the use of teflon or polypropylene tubes leaching of polychlorinated biphenyls (PCB's) may occur, which may affect experimental results, since they have similar properties to insecticides, and are toxic to aquatic organisms (Mayer *et. al.*,1977). Also, adsorption of the toxicant onto the aquarium surface is a possibility. Adsorption may also be a problem with pesticides and glass aquaria (Breach, pers. comm.). The plastic aquaria used, however, were not new and had been soaked in the outside holding tanks prior to use. They were divided into six enabling each tank to hold six animals, the division being necessary to prevent cannibalism of moribund animals (due to the effect of the toxicant). Also interaction between the crayfish may have caused increased stress thus affecting experimental results. The approximate 'floor' area of each division was 80 cm^2. Each tank was filled with 5 l of stock tank water, delivered from the outlet of the activated charcoal filter, 5 l being a convenient volume enabling easy calculation for the addition of toxicant.

At this stage the experiment to be conducted begins once the toxicant is added to the water. In order that all experiments involving toxicological tests may be directly comparable it is important that equivalent experimental procedures are conducted by all those concerned (e.g. see Adema, 1978). A particularly useful reference source outlining bioassay procedures for use in evaluating the toxicity of wastes and other water pollutants is Dourdoroff *et. al.*,(1951), and these guidelines have been adopted as nearly as possible by this author. They describe a toxic action as being 'any direct action of pollutants, including both internal

and external action', to be distinguished from indirect effects resulting from lowered oxygen levels, for example. Thus to detect direct action, near normal conditions and constant experimental procedures must be employed.

Static tests were conducted rather than 'flow-through' due to the problems of ensuring an accurate and sustained supply of a certain concentration of toxicant, and also the removal of large volumes of 'polluted' water. The use of polypropylene or teflon connecting pipes in flow-through systems can also be a problem due to the leaching of PCB's. Warlen and Engel (1978) have evaluated flow-through versus static bioassay systems and conclude that the former should be used wherever possible since a reduction in the toxicity of toxicants is observed in static bioassay systems, probably due to a reduction of the fraction of biologically available toxicant in the water due to adsorption and, with heavy metals (copper was used), complexation by biologically generated compounds. However, it is also true that the majority of tests are conducted in static systems, and very few in flow-through systems. For example, in a review of the literature on the pesticide HCH only one test is quoted as using a flow-through system whilst the remainder employed static systems (Macek *et. al.*, 1976). In order to ensure that the levels of toxicant in the tanks were as nearly as possible those desired, the water and toxicant were replaced daily using freshly made stock toxicant, and water from the stock tank.

As previously mentioned, the toxicants chosen for study were Lindane (λHCH, a pesticide) and cadmium (a heavy metal, see 1). Where concentrations of Lindane and cadmium are given

in the text it should be read that they have been made up from Gammalin (20% Emulsifiable concentrate) and BDH Cadmium Chloride crystals (99% pure) respectively, and in the following manner;

Lindane

100 mls of 100 p.p.m. stock solution was prepared thus;

0.001 ml Lindane per 1000 ml H_2O ≡ 1 p.p.m.

∴ 0.0001 ml Lindane per 100 ml H_2O ≡ 1 p.p.m.

and, 0.01 ml Lindane per 100 ml H_2O ≡ 100 p.p.m.

but, Gammalin is an emulsifiable concentrate containing 20% Lindane,

so, 0.05 ml Gammalin per 100 ml H_2O ≡ 100 p.p.m.

Thus the stock solution was prepared using 100 ml of deionized water measured into a graduated glass flask which had previously been rinsed with acetone to remove any grease to which the pesticide may adhere, and with dilute nitric acid to remove any other impurities adhering to the glass. 50 µl of Gammalin was dispensed into the 100 mls of water using a microliter pipette. A fresh stock solution was prepared for each experiment. The actual concentrations desired were achieved by pipetting the stock solution directly into the experimental tanks, for example, 5 litres of water require 5 mls of 100 p.p.m. stock solution to achieve a concentration of 0.1 p.p.m.

Cadmium

A high concentration stock solution of cadmium chloride was not prepared since initial tests using the atomic absorption spectrophotometer revealed that the concentration of cadmium in solution decreased with time, and it was felt that there may also be a problem with cadmium precipitating out of solution.

Thus, fresh preparations were made on each occasion;

1 mg of cadmium ions per litre of water ≡ 1 p.p.m.

and, 5 mg of cadmium ions per 5 litre tank of water ≡ 1 p.p.m.

but, cadmium chloride is only 49.221% cadmium by weight,

so, 5 mg of cadmium chloride per 5 litres of water ≡ 0.49221 p.p.m.

It may be seen therefore that x mg of cadmium chloride in 5 litres of water results in a cadmium ion concentration of approximately $\frac{x}{10}$ p.p.m. Thus if the desired concentration was 5 p.p.m., then 50 mg of cadmium chloride would be required. The actual quantities used were measured accurately to 0.0001g and then the precise concentration expected, using a given weight of cadmium chloride, was calculated. In certain cases the concentrations achieved were checked by atomic absorption spectrophotometry (see 4.1).

Controls

Control experiments were run concurrently with those employing toxicant although no Lindane or cadmium was added. The controls for comparison with the Lindane trials had an amount of acetone added to the water equal to the amount of Gammalin that would be present in the highest concentration of Lindane used. This was to allow for the emulsifiable concentrate, the precise composition of which was not known, but which would have consisted of an organic solvent carrier or carriers.

In each experiment only intermoult animals were used. Dourdoroff (1951) states that at least 10 animals should be used at each concentration for each test. However, owing to the large number of animals this would require in order to achieve more than just

one set of experimental results, the usual number of crayfish used was 6 (i.e. one tank) per concentration used per trial. Greater statistical validity would have been achieved by using more animals, but at the expense of the number of different trials it would have been possible to conduct.

When it was necessary to kill a crayfish, freezing in a small plastic bottle was the method employed and was considered the most humane method possible without unduly altering the tissues of the animal. On chilling, the crayfish very quickly become moribund. The respirometry experiments involved excising live tissue from the live animal which was immediately killed after dissection by immersion in boiling water. To reduce stress, dissection of live animals was conducted only after they had been chilled, in very cold water, to inactivate them.

The dry weight of various tissues was obtained by dissecting them out into preweighed and numbered glass vials (10 ml capacity), and then drying to a constant weight in an oven set at 60°C containing a desiccant, usually for 48 hours. The vials and dried tissue were then cooled over a desiccant prior to weighing accurately to 0.0001g. The dry weight of tissue was obtained by substraction.

CHAPTER 3

THE TOXICITY OF VARIOUS TOXICANTS TO *A. PALLIPES*

3 (i) INTRODUCTION

There is a vast array of information relating to the effects of both heavy metal and pesticide toxicants on aquatic organisms. Rather less relates specifically to the acute toxic effects of these toxicants to freshwater crustaceans, and in fact very little exists relating to the toxicity of cadmium despite a large number of papers relating to its uptake into the tissues of Crustacea. More information is available for Lindane, although the author is only aware of one other paper which reports the toxicity of Lindane to a crayfish species (see 3(iv)), and most of the data relating to crayfish deals with other insecticides. This study hence presents a valuable contribution towards the study of the toxicity of both cadmium and Lindane to freshwater crustaceans, and in particular to *A. pallipes* for which no other similar studies have been conducted.

Increasing industrialization which has lead to an increase in the levels of cadmium occurring in natural waters, and the increasing use of agrochemicals, which through agricultural run-off increase the aquatic loading of certain pesticides, including Lindane (see 1), and the growing awareness of the effects of these toxicants on aquatic life, emphasises the need for information upon which to base water quality criteria, and safe levels of toxicants. Crustaceans appear to be more sensitive to both heavy metal and pesticide toxicants than other aquatic organisms (e.g. for reviews see: Heavy metals - Thorpe *et. al.*, 1979; Pesticides - US EPA-440, 1980), and so form a logical group of animals upon

which to base and assess existing water quality criteria. *A. pallipes* is one such organism, and both juvenile and adult stages of the life cycle are examined.

The use of experiments to assess median lethal doses (LD_{50}) of toxicant required to kill an organism in a certain time (usually 96 hours), which although limited in their comparability with the field situation, are nevertheless useful, and provide a valuable index of the relative toxicities of different toxicants, and the relative toxicity of single toxicants to different organisms. It is also possible to determine harmless concentrations of toxicants based upon the results of such studies (3(iv)). LD_{50} tests, however, have been criticised (e.g. Sharpe, 1981) and it is true that generally they relate to very specific experimental conditions. Chronic rather than acute tests, it is argued, should be conducted (e.g. Sprague, 1971) since these bear more relation to the levels of pollutants which may be found in the environment. However, this chapter assesses the LD_{50} 96 hour values of cadmium and Lindane to *A. pallipes*, and it is argued that this is justifiable both as a means of comparison with other species, and as a means of assessing what a sublethal level of toxicant may be. It is all very well to expound the values of chronic tests, but before it is known what levels of a toxicant are lethal to an organism, the concentrations to use in chronic tests cannot be gauged accurately.

3 (ii) MATERIALS AND METHODS

Short term acute toxicity tests (LD_{50} 96 hours) were conducted on both the juvenile and adult stages of *A. pallipes*. Those animals surviving beyond 96 hours were also monitored in order to determine

the median lethal time of exposure (TLM_{50}) for each concentration. In general the procedures outlined by Dourdoroff *et. al.*, (1951) were used as a basis for these tests, and the methods described previously (2) were employed.

ADULTS

Nanpantan stock were used for tests employing Lindane as the toxicant, whilst Markfield stock were used for the cadmium tests. Only adult intermoult males were used (eliminating the possibility of sexual differences) of mean size 28.1 ±1.8 mm (Carapace length) from the Nanpantan stock, and 35.4 ±1.1 mm (C.L.) from the Markfield stock. Crayfish were exposed in groups of six to a range of concentrations of toxicant in a previously aerated static system. Controls were treated similarly, but were not exposed to toxicant (see 2). The experiments were conducted in a coldroom maintained at 10 ±1°C, and the range of concentrations of toxicant employed, in p.p.m., was;

Lindane, 0(control),0.005, 0.025, 0.05, 0.25, 0.5

Cadmium, 0(control), 10, 20, 30, 40, 50

Water and toxicant were changed daily up to 4 days (96 hours), and twice weekly thereafter. No test continued for longer than 31 days.

The criterion of lethality was chosen to be mortality in both cases, i.e. when no response was elicited when animals were prodded with a glass rod, and all pleopodal and mouthpart movements had ceased. Loss of equilibrium often occurred long before death, however, and has sometimes been employed as the criterion of lethality (e.g. see Canton *et. al.*, 1975; Canton and Sloof, 1977). This method may have been possible in the Lindane tests, although

observations indicate that equilibrium is sometimes regained with increased time of exposure, but loss of equilibrium is more difficult to detect in the case of cadmium. Mortality was recorded every 3 hours for the first 96 hours (except between 00.00 and 06.00 hours), and daily thereafter.

JUVENILES

Nanpantan stock of mean size 5.2 ± 0.3 mm (C.L.), reared at Nottingham University were used for the tests employing Lindane as the toxicant. It was not possible to obtain Markfield Quarry juveniles to enable direct comparisons with the adult trials, so juveniles netted from the River Leen, of mean size 5.6 ± 0.4mm (C.L.) were used for the tests employing cadmium as the toxicant. Environmental conditions of light and temperature were identical to those employed for tests with adult crayfish. The experiments were conducted in plastic petri dishes containing a square of nylon mesh for the juveniles to cling to in the case of the Lindane trials. The cadmium experiments employed 10 x 10 x 20 cm plastic containers with the bottoms cut out and replaced by nylon mesh. These were placed within the usual plastic aquaria containing 5 litres of water and toxicant. Juvenile crayfish were exposed in three groups of five to each of a range of concentrations of Lindane whilst only six animals were used at each concentration for the cadmium trials. Controls were treated similarly but were not exposed to toxicant. The range of concentrations of toxicant employed, in p.p.m., was;

Lindane, 0(control) 0.0005, 0.0025, 0.005, 0.025, 0.05, 0.1, 2.5, 5.0, 10.00

Cadmium, 0(control) 0.5, 1.5, 3.0, 4.0, 5.0, 10.0, 50.0

N.B. Pre-experimental trials to gauge the range of toxicants to use employed fewer animals, viz., Lindane 5 p.p.m., 10 p.p.m. - 6 animals, Cadmium 10 p.p.m., 50 p.p.m. - 3 animals.

Water and toxicant were replaced daily, and no test was continued for longer than 10 days. The criterion of lethality was mortality, which was monitored every 3 hours up to 96 hours, and daily thereafter. The trials involving juvenile mortality to cadmium were ceased after day 5 for all concentrations above 3 p.p.m., by which time TLM_{50} values had been achieved, and the survivors returned to freshwater. None survived beyond day 3.

Results for both adults and juveniles were analysed using a modified version of the method of Lichfield and Wilcoxon (1949) for the analysis of dose effect experiments. A typical dose effect curve is sigmoid in shape and after being linearized may be modelled by the application of regression analysis (Whitehead, 1980). Lichfield and Wilcoxon's (1949) method is a simplification of probit analysis and involved plotting the percentage mortality at 96 hours on a probability (Probit.) scale against the concentration of Lindane on a logarithmic scale. The LD_{50} 96 hour value and its 95% confidence limits (2½ S.D., equivalent to the concentrations causing 16% and 84% mortalities) may be estimated from the best fitting line. However, rather than fitting the line by eye, the test concentrations and percentage mortalities were converted to logs. and probits. respectively and the best fitting linear regression line was calculated, using regression analysis. The LD_{50} 96 hours occurs when the probit. percentage mortality is 5.00, i.e. 50%, and 95% confidence limits may be

calculated by substituting the values of 4.01 (16%) and 5.99 (84%) for probit. percentage mortality into the equation calculated by regression analysis. Zero and 100% mortalities do not convert directly into probits, and yet these results are significant and deserve inclusion in the data. As a consequence Lichfield and Wilcoxon (1949) provide a table of corrected values for 0% or 100% mortalities relating them to corresponding expected values, and describe how these may be obtained. Hence it was possible to include all of the data in the analysis of results.

A log. linear model has been employed to describe the relationship between TLM_{50} and toxicant concentration, and LD_{50} 96 hour values are also derived.

ADDITIONAL DATA

During the execution of the acute toxicity tests certain additional data was generated for a variety of reasons, and although they do not constitute individual experiments, the results obtained are worth mentioning.

Concurrent with the trials on the effects of Lindane to juvenile *A. pallipes*, additional trials using juveniles from the same source, and employing identical experimental procedures, were conducted using Permethrin (a pyrethroid insecticide), and Malathion (an organophosphate insecticide). The concentrations employed, in p.p.m., were;

Permethrin, 0(control), 0.0005,0.0025,0.005,0.05,0.5,1.0,2.5, 5.0,10.0

Malathion, 0(control), 0.0005,0.0025,0.005,0.05,0.5,1.0,2.5, 5.0,10.0

These trials employed the same control animals as for those on

Lindane toxicity. Further tests using these two toxicants were not conducted, and experiments were confined to Lindane for reasons outlined previously (1, 2).

The trials conducted with Markfield adults for cadmium toxicity, described above, relate to the final regime employed. Initially identical experimental procedures had been followed using Nanpantan stock (C.L. 27.1 ± 0.6 mm) and the following range of concentrations, after consideration of the literature (e.g. Thorpe, *et. al.*, 1979);

Cadmium, in p.p.m., 0.25, 0.5, 2.5, 5.0, 10.0, 20.0, 30.0.

However, after 7 days no animals had died at any of the concentrations examined, and so these animals were incorporated into the uptake of toxicant tests (see 4.1). A second trial was established using more Nanpantan stock (C.L. 33.3 ± 1.2 mm) and using concentrations thus; cadmium, p.p.m., 50, 63, 75, 88, 100. After 96 hours no mortalities had occurred, so with the exception of those animals exposed to 100 p.p.m., the remainder were removed to fresh water, and their survival monitored.

That the Markfield Quarry stock were more sensitive to cadmium pollution became apparent during initial respirometry experiments, the concentrations for which had been chosen after the experience with Nanpantan stock.

3(iii) RESULTS

Tables 3.1 and 3.2 show the results of the toxicity trials on both juvenile and adult *A. pallipes* using Lindane and cadmium as the toxicant respectively. Table 3.3 shows the additional data generated relating to the toxicity of Permethrin and Malathion to juvenile crayfish. Analysis of these results by the modified method of Lichfield and Wilcoxon is presented in Table 3.4. LD_{50}

96 hour values are given, with 95% confidence limits, and regression analysis reveals that all results are significant to at least the 5% level. The LD_{50} 96 hour values were, in p.p.m. of toxicant; Lindane, juveniles, 0.12, adults, 0.48; Cadmium, juveniles, 4.14, adults, 36.45; Permethrin, juveniles, 0.03; Malathion, juveniles, 0.06. Analysis of the TLM_{50} data is presented in Table 3.5 and illustrated in Figs. 3.1 - 3.3. All results are significant to at least the 5% level, and with the exception of the result for juveniles exposed to Lindane, the LD_{50} 96 hour values calculated from this analysis correspond closely to those calculated by the method of Lichfield and Wilcoxon (1949). The result for juveniles exposed to Lindane, however, falls within the 95% confidence limits calculated in the former analysis. The LD_{50} 96 hour values derived by the latter method were, in p.p.m. of toxicant; Lindane, juveniles, 0.04, adults, 0.44; cadmium, juveniles, 3.98, adults, 30.36; Permethrin, juveniles, 0.03; Malathion, juveniles, 0.03. Regression analysis included only one set of data points which exhibited a TLM_{50} in excess of the experimental period for those cases where two sets of such data existed.

The LD_{50} 96 hour values show that Lindane is more toxic to *A. pallipes* than are cadmium ions for both adults and juveniles when a direct comparison is made between the concentrations used. For each toxicant the juveniles are more susceptible than the adults, and Figs. 3.1 - 3.2 show this to be the case throughout the full range of concentrations employed. A comparison of juvenile *A. pallipes* exposed to Lindane, Permethrin, and Malathion reveals that from the 96 hour LDso results, the order of toxicity is Permethrin > Malathion > Lindane. Fig. 3.3 reveals, however,

that at higher concentrations Lindane is the most toxic, whilst at very low concentrations juvenile *A. pallipes* are better able to tolerate Lindane than the other insecticides.

The 'symptoms' of poisoning for exposure to Lindane were initial stress exhibited by increased activity over that of the control animals. Loss of equilibrium followed and the crayfish would fall onto their 'backs' waving their legs around. Equilibrium was in some instances regained with continued exposure, but all animals exhibited sudden very jerky movements when disturbed. Juveniles tended to shed their chelae, possibly a predator escape response, whilst a very characteristic posture adopted by adult crayfish was to have the tail thrust straight back and to walk on 'outstretched' legs. The effects of cadmium poisoning were not nearly so obvious. There was no initial increase in activity compared with the controls, and loss of equilibrium did not consistently occur. When aroused, slow, 'deliberate' scrabbling movements of the legs occurred and was similar for both adults and juveniles. Juveniles did not tend to shed their chelae. Dead animals appeared to be very swollen, particularly at the junction between the carapace and abdomen. This may indicate a loss of osmotic control.

The results of the initial LD_{50} trials for cadmium using Nanpantan stock showed no mortalities after 96 hours (see 3(ii)), and except for animals exposed to 100 p.p.m. cadmium, crayfish were returned to fresh water. All died within 17 days in fresh water, whilst the controls remained alive, and the evidence did not suggest that those exposed initially to the highest concentrations died more quickly than the rest. Juvenile crayfish initially

exposed to cadmium ions and then returned to fresh water also died (within 3 days). For those crayfish exposed to 100 p.p.m. cadmium no mortalities occurred until day 7, but all had died by day 11. At these very high concentrations, however, the water assumed a milky appearance, and it may be that due to precipitation the levels of cadmium biologically available were less than the nominal concentrations. Problems with the solubility of cadmium chloride at high concentrations have been reported elsewhere (e.g. Club *et. al.*, 1975).

3(iv) DISCUSSION

The results show that from the concentrations of toxicant required to kill both adult and juvenile crayfish, Lindane is more toxic than cadmium by a factor of 35 (juveniles) - 75 (adults). This is to be expected, however, since Lindane is a man-made substance designed specifically to kill insects, which are phylogenetically close to the Crustacea. By contrast, even for the most toxic metals (cadmium is rated as a heavy metal of medium toxicity, see 1) relatively large amounts are required to cause acute toxic effects, and on the relative logarithmic scale of toxicities metals do not occupy a high position. This scale is expressed in pT values, where pT is - log. lethal dose, expressed in mol.Kg $^{-1}$, and the value for sodium chloride is 1.3, for heavy metals 4-5, for Dioxin 8, and for the most lethal natural toxins 15 (Piotrowski and Coleman, 1980). No value is given for pesticides, but it must be at the upper end of the scale considering their highly specific properties. As a consequence pesticides are to be considered of more concern than heavy metals when examining the dangers of acute toxicity resulting from any pollution, whilst

the chronic toxicity of heavy metals which may occur after long term exposure of an organism to low levels of toxicant is of more concern in the latter case.

That cadmium ions are the toxic element in cadmium chloride has been established using chlorides of other less toxic metals such as sodium (e.g. Anderson, 1946; Club *et. al.*, 1975). The toxic effects may appear at the molecular level causing biochemical damage, or at cellular and higher levels of organization, and ultimately may lead to physiological impairment and death. The toxicity may also be influenced by other elements in the environment (e.g. Sprague, 1970). That cadmium is taken up into the tissues of the crayfish, particularly the gills, will be established (see 4.1), and also that respiration is affected (see 5), and its toxic effect has been demonstrated. The precise cause of death of crayfish exposed to cadmium ions is probably due to loss of respiratory or osmoregulatory function at the gills, or to loss of both. The gills of crustaceans are particularly important for osmoregulation due to the exoskeleton which acts as an impermeable barrier to the transport of water and ions (Sutcliffe, 1978). Additionally, cadmium has also been seen to influence protein and nucleic acid synthesis in the rat, certain hepatic enzymes in fish, and leads to a reduction of the protein content of chironimid larvae (Rathore *et. al.*, 1979). Blundell and Jenkins (1977) suggest that the toxicity of heavy metals may be due to them binding with proteins, whilst metal binding proteins may help to offset the toxic effects of heavy metals in conditions of chronic low level exposure (Thorpe *et. al.*,1979). Indeed, evidence for cadmium binding proteins has been found

in Blue crabs environmentally exposed to cadmium (Wiedow *et. al.*, 1982). In acute toxicity tests such as that described above (3(ii)), death may not be directly related to the toxic action of the heavy metal, but more due to excessive stress being placed upon the natural mechanisms of the organism due to the extremely high and unnatural levels of toxicant. The toxic stress resulting may involve an increased demand for enzyme production, and possibly also the loss of ligand sensitivity, by which enzyme regulation rates are controlled (Calabrese *et.al.*,1977).

It may thus be seen that cadmium has a variety of effects on the physiology of an organism. The conclusion that the major cause of death was loss of respiratory and/or osmoregulatory function derives from the fact that the gills appear to be the chief site of uptake of cadmium (4.1). Additionally, cadmium poisoned animals were observed to be swollen in appearance (3(iii)), possibly indicating a loss of osmoregulatory function. A similar conclusion was the result of cadmium toxicity tests on the marine crab *Uca pugilator* (Vernberg *et. al.*, 1974). Cadmium is also seen to be taken up by the gills of other decapod crustaceans and examination by transmission electron microscopy shows extensive areas of cell death (e.g. marine shrimp - *Penaeus duorarum* (Couch, 1977); fresh-water prawns - *Machrobranchium* and *Caridina* (Ghate and Mulherkar, 1979)). Similar electron microscope studies conducted by this author with gills from *A. pallipes* exposed to cadmium revealed no differences from the control animals, and no obvious signs of cell death were apparent despite the relatively high concentration of cadmium in the tissue. Couch (1977) suggests that the necrosis observed in gills of *P. duorarum* could cause osmoregulatory and

respiratory dysfunction, and despite the lack of obvious signs of necrosis in gills of *A. pallipes* it is likely that the cadmium would have a similar effect. The uptake of cadmium by the amphipod *Gammarus pulex* has been shown to be active, and may take place via the route normally used to take up calcium, calcium influx being inhibited by the presence of cadmium ions (Wright, 1980). Within the crayfish gill tissue cadmium affects the rate of respiration (5) probably since it affects enzymes necessary for respiration. In the rock crab cadmium has been shown to cause inhibition of sodium and potassium ATPase in the gill tissue (Tucker and Matte, 1980). Thus the metal will affect enzymes in both the mitochondria and cellular membranes causing adverse effects to the osmoregulatory and respiratory functions of the gills ultimately leading to death. The methods by which heavy metals may alter enzyme activity have been described by Tucker and Matte (1980) and include:

(i) Binding to side chains of enzymes such as imidazol, histidyl, carboxyl and sulphydryl.

(ii) Binding at or near enzyme active sites thus causing interference with substrate binding.

(iii) Conformational changes of the active site may occur even when metals bind at a point away from the active site.

(iv) The heavy metal may replace a normal co-factor, especially in ATPases (e.g. Mg^{2+}).

(v) Metals may simply change the concentration of co-factors or reactants (as described above for calcium) by altering membrane permeability.

The toxicity of Lindane may also be attributed to a variety

of factors, and its biological properties have been reviewed by Demozay and Marechal (1972c). In insects it acts as a neurotoxin and respiratory poison. Intoxicated insects show atoxia and convulsions which in the extreme case may lead to death by paralysis. In cockroaches and beetles it appears to act directly at the nerve ganglion and the effect is manifest in physiological disturbances such as dehydration, raised oxygen consumption, and powerful inhibition of certain respiratory enzymes such as cytochrome oxidaze and succinodehydrogenase. Cellular breakdown and the loss of nuclei has also been demonstrated in insects after exposure to Lindane.

The effects of Lindane on the respiration of *A. pallipes* will be reported (5), and its sites of uptake will be established (4.2), and shown to be chiefly the hepatopancreas, and similar loss of muscle control, convulsions and jerky movements to those of insects have been reported as being the effect of Lindane on *A. pallipes*. It would appear therefore that the mode of action of the insecticide could be similar in crayfish and insects. Lowe (1964) also reports paralysis of crustaceans through exposure to pesticides, including Lindane, and generally it is found that crustaceans are more susceptible to Lindane than other forms of aquatic life (see Bodenstein, 1972; US EPA-440, 1980, for reviews). This is to be expected since being arthropods they are phylogenetically close to the insects, and insecticides are selected specifically for their ability to kill certain groups of insect pests.

Death of *A. pallipes* during the LD_{50} 96 hour tests therefore could have been due to paralysis and nervous disorder accompanied

by respiratory and osmoregulatory dysfunction. Tests conducted on the Blue crab, *Callinectes sapidus*, using low level doses of another organo-chlorine insecticide, D.D.T., however, have shown that sodium and potassium A.T.Pase activity is initially inhibited. Subsequently, and quickly, either (i) rapid induction or activation of new Na, K-A.T.Pase activity occurs, or (ii) excess sodium transport capacity naturally available, allows for increased sodium movement, and the result is that the crab is protected from osmoregulatory difficulty (Neufeld and Pritchard, 1979a; 1979b). Even if a similar mechanism operated in *A. pallipes* with regard to Lindane, the high levels of toxicant employed would be sufficient to 'overload' the natural mechanisms. Evidence for the activity of organochlorine insecticides on the nervous system of crayfish has also been established (Shrager *et. al.*,1969).

In mammals high levels of Lindane in the tissues lead to stimulation of greater activity of microsomal enzymes in the liver, increasing metabolism and excretion of the toxicant (Herbst and Bodenstein, 1972). D.D.T. has a similar effect in mammals, but no evidence for the induction of microsomal enzymes in the crayfish *Astacus astacus* has been found (Lang *et.al*,1976), although the hepatopancreas of the Blue crab has been shown to be capable of metabolising D.D.T. to its metabolites (Neufeld and Pritchard, 1979b). The lack of induction of mixed function oxidase enzymes in the hepatopancreas of *A. astacus* which help in detoxification may mirror the situation for *A. pallipes* and Lindane, and may explain the high degree of sensitivity to organochlorine insecticides reported for crustaceans. Even at low concentrations insufficient to cause mortalities Lindane has been shown to interfere with

the reproduction of both molluscs and crustaceans (Bodenstein, 1972; Bluzat and Seuge, 1970).

The results of this study showed that juvenile crayfish were less able to tolerate toxic stress than adults, and the lethal concentrations were less by a factor of 10 for both Lindane and cadmium (4 for Lindane if the results of the method of Lichfield and Wilcoxon are used). The results of the TLM_{50} study, illustrated in Figs. 3.1 and 3.2, showed that the heightened sensitivity of juveniles to toxicant was consistent throughout the full range of concentrations employed, and showed that adults are better able to tolerate a low level of toxicant pollution and may survive where juveniles would be killed. This is of significance to the natural situation since it shows that although low levels of toxicant may not kill adult crayfish, a population may still be destroyed due to interference with reproduction, and to the heightened susceptibility of juveniles to low levels of toxicant. The increased susceptibility of juvenile stages of the crustacean life cycle over adult stages have been reported elsewhere, and are of a similar order, for both heavy metals, including cadmium (e.g. Cadmium - *Uca pugilator* (Vernberg *et. al.*, 1974); Copper - *Procambarus clarkii* (Hobbs and Hall,1975); Mercury - *P. clarkii* and *Faxonella clypeata* (Heit and Fingerman, 1977)), and pesticides, including Lindane (e.g. Malathion - *Orconectes naiis* (Sanders, 1972); Lindane - several species reviewed (US EPA-440, 1980); Permethrin - *P. clarkii* (Jolly *et. al.*, 1978)). Such results illustrate the need for care when comparing like experiments, and it must be ensured that a similar size-group of animals is compared and that the stage in the life history is documented.

The age of an animal, however, is evidently not the only criterion which affects susceptibility to toxicity. This was apparent when it was observed that *A. pallipes* from the Markfield Quarry population were more susceptible to cadmium poisoning than those from the Nanpantan population. The explanation may lie in the fact that the Nanpantan stock had previously been exposed to low levels of heavy metal pollution, possibly including cadmium, which had resulted in biological adaptation lessening the effects of the toxicant. This could include metal binding proteins which were activated when crayfish were exposed to cadmium ions. Such biological adaptation can occur for heavy metals (Piotrowski and Coleman, 1980), and has also been observed in crayfish exposed to low levels of insecticide pollution (Albaugh, 1972). It was not possible to measure the levels of heavy metals in Nanpantan Reservoir, however, since the reservoir was drained in 1979 (see Part I, 2.1(iv)), and also no water authority data was available for that year. Data was available up to 1978 although cadmium was not specifically measured, and the maximum levels of certain metals reported in the period 1976 - 1978 were; Lead, <0.01p.p.m., iron, 0.22 p.p.m., copper, 0.08 p.p.m., and manganese, 0.16 p.p.m. (S.T.W.A. Divisional Scientist, pers. comm.). The levels of heavy metals in Markfield Quarry water were measured on a single occasion by the author (4.1) and were Lead, <0.01 p.p.m., iron, 0.20 p.p.m., copper, 0.01 p.p.m., manganese, 0.04 p.p.m., zinc, 0.01 p.p.m. and cadmium < 0.01 p.p.m. It will be seen that the concentration of metals in each water body were of the same order of magnitude, although slightly higher concentrations of iron, copper, and manganese occurred in Nanpantan Reservoir.

In addition to the intra specific variations in susceptibility to toxicity described, interspecific variations also occur. Crustaceans tend to be more susceptible to both heavy metal (e.g. Thorpe *et. al.*, 1979) and insecticide (e.g. Macek *et. al.*, 1976) pollutants than many other aquatic organisms, including fish. This is of importance since crustaceans often form an important part in any food web and so their elimination could alter the ecology of a water body, and indirectly affect other organisms which the toxicants had not directly affected. The most valuable comparisons to be made are thus with similar toxicants and their effects on crustaceans, particularly crayfish.

Despite a relatively large literature on the uptake of cadmium into the tissues of crustaceans, very little information exists concerning lethal concentrations of this metal, particularly with freshwater organisms. A summary of the relevant information is presented in Table 3.6 and includes some data on the lethal dose of cadmium to freshwater insect species, owing to the lack of data for freshwater crustaceans. Compared to the other freshwater crustaceans for which LD_{50} values are given (the Daphnids), *A. pallipes* is seen to be far more tolerant of cadmium ions. The other crayfish species mentioned were in chronic tests at very low concentrations of cadmium. Adult *Orconectes propinquus* survived in 1 p.p.m. of cadmium ions for up to 190 days (Gillespie *et. al.*, 1977)), and *A. pallipes* maintained at similar concentrations for uptake studies (4.1) survived for up to four weeks. *Cambarus latimanus*, however, seemed more sensitive and out of 48 test animals, 2 at 0.01 p.p.m. died within two weeks, and 1 at 0.005 p.p.m. died within nine weeks (Thorpe *et. al.*, 1979), although possibly

these animals may have died anyway. By comparison with *O. propinquus* then, the LD_{50} 96 hour values for *A. pallipes* do not seem excessively high, and indeed by comparison with the insects reported, which are considerably smaller than crayfish, similar values for LD_{50} 96 hours are seen to exist. The results are also of the same order as reported for the marine crabs, *Uca pugilator* and *Eurypanopeus depressus*, although the shrimp, *Paratya tasmaniensis* is considerably more sensitive to cadmium ions than either *A. pallipes* or the other marine crustaceans reported (Table 3.6). For pesticides it is certainly the case that marine organisms are more sensitive to toxicants than are freshwater organisms, although this is not immediately apparent as being the case with cadmium and Crustacea. However, Vernberg *et. al.*, (1974) report that the toxicity of cadmium to *U. pugilator* is both temperature and salinity dependent. Crabs were more susceptible to cadmium under conditions of high temperature and low salinity than the opposite and so under normal conditions (of high salinity) it would appear that the levels of cadmium that the marine crabs could tolerate were of a similar order to the levels tolerated by adult *A. pallipes* (46.6 p.p.m. *U. pugilator* cf. 36.5 p.p.m. *A. pallipes*). The other marine organisms were more sensitive to cadmium poisoning.

Regarding the toxicity of Lindane in acute tests, there is rather more information relating to crustaceans than with cadmium. A summary of relevant data is presented in Table 3.7. It may be seen that of the freshwater crustaceans, the isopod *Assellus brevicaudus* and amphipod *Gammarus fasciatus* are the most sensitive to Lindane, although *Gammarus lacustris* requires 48 times the amount of toxicant required to kill half of the

G. fasciatus in 96 hours, so results may not always be consistent within genera. Similarly when comparing the two crayfish species *Astacus leptodactylus* and *A. pallipes* it is found that the former is 10 times more sensitive to Lindane, and the LD_{50} 96 hours value for adult *A. leptodactylus* corresponds more closely to that of juvenile *A. pallipes*. A similar LDso 96 hours value to adult *A. pallipes* was reported for the cladoceran *Daphnia pulex* whilst *D. magna* was slightly more sensitive. Note also the greater toxicity of Lindane, the λ isomer of BHC, compared to αHCH(BHC) (see 1).

The greater sensitivity of marine Crustacea to Lindane is immediately apparent from Table 3.7 despite the considerable variation of toxicities attributed to the different shrimp species themselves. In the extreme, Lindane is 2,800 times more toxic to *Penaeus duorarum* than *A. pallipes*!, although it must be noted that the least sensitive shrimp species differs from *P. duorarum* by a factor of 58 itself. The heightened sensitivity of marine species may be explained by the fact that they have a greater problem in maintaining osmoregulation than freshwater species. Thus if Lindane interferes with osmoregulatory processes at the gills, the marine organisms will immediately be under more environmental stress, and will thus be more sensitive to Lindane.

The concentrations of both cadmium and Lindane which occur in the rivers of the Severn-Trent Water Authority are given in Tables 1.1 and 1.2. The maximum concentration of cadmium recorded was 0.28 p.p.m. in the Leen, after the confluence of the two branches, although this result may be too high (see 4.1). Below this value, the next highest was 0.02 p.p.m. cadmium ions, detected

in the River Erewash. These concentrations are sufficiently high to kill *Daphnia* spp. and are also high enough to cause bio-accumulation of the metal in crayfish (0.01 - 1.0 p.p.m., Gillespie *et. al.*, 1977; 0.25 p.p.m., Author, see 4.1), although tissue concentrations would not be very high since uptake is related to the external concentration of metal ions (4.1). The concentrations occurring in these rivers are considerably higher than expected for unpolluted fresh water bodies (< 1 ngl^{-1} i.e. 10^{-6} p.p.m., Piotrowski and Coleman, 1980) and may explain the loss of crayfish from some areas, although compared to the LD_{50} 96 hours values for cadmium and *A. pallipes* these concentrations probably only constitute a sublethal level. The toxic effect, however, may be enhanced by the presence of other pollutants (e.g. see Sprague, 1970). It will be noted also that in both Markfield Quarry and the population study area in the East branch of the Leen where crayfish populations exist, heavy metal concentrations were very low, and cadmium was beyond the limits of detection.

Dourdoroff *et. al.*, (1951) make the point that LD_{50} values are only an index of the relative toxicity of a toxicant under defined conditions, and that for purposes of recommending safety limits for toxicant disposal, dilutions must be made. Harmless concentrations may be derived from LD_{50} data thus;

$$C = \frac{48 \text{ hr. } LD_{50} \times 0.3}{S^2}$$

where, 'C' is the harmless concentration,

and, $$S = \frac{24 \text{ hr. } LD_{50}}{48 \text{ hr. } LD_{50}}$$

Using the models which describe the relationship between

cadmium concentration and TLM_{50}, (the time at which 50% mortality occurs at that concentration), LD_{50} 24 hour and LDso 48 hour values may be calculated. They may also be read directly from Fig. 3.2. Thus, using the above formula, concentrations of cadmium which are harmless to juvenile *A. pallipes* are 0.52 p.p.m., and for adult crayfish are 3.52 p.p.m. It is suggested that these 'safe' concentrations are perhaps a little optimistic despite the fact that juveniles kept in concentrations of 0.5 p.p.m. cadmium survived for 7 days during toxicity trials, and adults maintained in concentrations of 5 p.p.m. survived for 4 weeks during cadmium uptake trials, but based on these values the concentrations of cadmium occurring in the rivers of the Severn-Trent area are safe to *A. pallipes* in the absence of any other toxicants. One other point of interest from these studies is that in support of the arguments for the lack of crayfish in the Leen after the confluence with the West branch (see Part I, 3.3(i)), Maciorowski *et. al.*, (1980) report avoidance reactions to cadmium ions by the crayfish *Cambarus acuminatus*. Crayfish avoid concentrations of 0.125 p.p.m. cadmium ions, and if similar for *A. pallipes* concentrations of cadmium after the confluence of the two branches of the Leen may be sufficient to elicit avoidance reactions.

The concentrations of Lindane which are found to occur in the Severn-Trent area reached a maximum of only 0.00052 p.p.m. in 1978 (Table 1.2) and this was in the River Erewash. By comparison with the results in Table 3.7, such concentrations do not pose a threat to freshwater crustaceans. Harmless concentrations of Lindane calculated in the same manner as for cadmium are 0.0051 p.p.m. for juvenile *A. pallipes* and 0.041 p.p.m. for adults, and

so the levels of toxicant in the environment are well within the safe limits for *A. pallipes*, and also those recommended by the U.S. Environmental Protection Agency of 0.002 p.p.m. (see 1). No data was available for the River Leen, but in the Vale of Evesham some Lindane is detectable in the rivers as agricultural run-off (Breach, pers. comm.). The level of crop spraying in the area of the Leen is likely to be well below that in the Vale of Evesham, and none should occur at Markfield Quarry. Concerning the harmless concentrations calculated for both cadmium and Lindane, it will be seen that what is regarded as harmless to adult crayfish is still within the range of concentrations toxic to juveniles (Table 3.4). This reinforces the point that when devising safe limits for toxicants the most susceptible stages of the life history of an organism must be the basis of these limits, and ideally the most susceptible of a range of organisms should be used.

Using the most susceptible stage of the life history of *A. pallipes*, the juveniles, the toxicity of Permethrin and Malathion was also determined. Permethrin is a synthetic pyrethroidinsecticide which is highly toxic to insects, but not very toxic to mammals (Jolly *et. al.*, 1978; Muirhead-Thompson, 1978). Malathion is an organophosphate insecticide which acts by inhibiting esterases. Thus organophosphate insecticides act as nerve poisons by blocking synaptic transmission in the cholinergic parts of the nervous system (Coppage and Mathews, 1974). A comparison of the toxicities of these insecticides with Lindane (an organochlorine insecticide) showed that the LD_{50} 96 hour values were all very similar, but that at more dilute concentrations both Permethrin and Malathion

were more toxic to juvenile crayfish than Lindane, Malathion being the most toxic (Fig. 3.3). Table 3.8 compares the toxicities of these insecticides to other freshwater crustaceans, mostly crayfish species.

From Table 3.8 it may be seen that a range of concentrations of each insecticide are required to kill different organisms. A comparison of the concentrations of Lindane required has already been discussed. For Permethrin it may be seen that the most sensitive organism is *Gammarus lacustris*, but that other amphipods are considerably less sensitive. Similarly for juvenile crayfish, with *Procambarus clarkii* being 76 times more sensitive than *A. pallipes*. The range of toxicities exhibited by Malathion is equally broad, the amphipods being the most sensitive whilst adult crayfish apparently show no signs of 'toxic effects or mortalities at concentrations as high as 100 p.p.m. Comparing juvenile crayfish, *A. pallipes* is considerably more sensitive to Malathion than *Procambarus acutus acutus* and its LD_{50} 96 hour value corresponds more closely with the freshwater shrimp *Palaemonetes kadiakensis*. A comparison of the relative toxicities of the insecticides has put all three as of approximately equal toxicity to *A. pallipes* at 96 hours LD_{50} values, but at lower concentrations the order of toxicity is Malathion > Permethrin > Lindane. Since comparison between insecticides may be made safely only within single species, due to the great intraspecific variations observed, the only other comparable results are those relating to *Gammarus fasciatus*, *Gammarus lacustris* and *Daphnia magna*. In the former case the order of toxicities was Malathion > Lindane > Permethrin, with Lindane and Permethrin being almost equal. In the second case

Permethrin is considerably more toxic than Lindane, and in the last example, Malathion is seen to be more toxic than Lindane. Overall then, the relative toxicities of the insecticides would appear to be that reported above for *A. pallipes*. Sanders (1969) concluded that organophosphate insecticides were the most toxic, though only slightly more so than organochlorine insecticides and Muncy and Oliver (1963) also found an organophosphate insecticide to be the most toxic of 10 they tested, although as reported in Table 3.8, they found Malathion not to be toxic to *P. clarkii* at 20 p.p.m.. Malathion was also found to be the least toxic insecticide tested on three out of four freshwater crustaceans, the exception being *G. fasciatus*, as reported (Sanders, 1972).

Data additional to the LD_{50} 96 hour values of various toxicants which was generated related to the transference of cadmium exposed animals to clean water. Both juvenile and adult crayfish were involved, and all test animals eventually died. The implications of such an observation are that although high levels of pollutants are not generally found in naturally occurring water bodies, a short term flush of high concentrations of pollutants such as cadmium, following an accidential discharge for example, could be sufficient to destroy crayfish populations despite the relatively rapid return to normal conditions. Tests conducted using Lindane and *Gammarus pulex* have also shown that short term exposure of the organism to sufficiently high concentrations of Lindane will kill them, despite a return to clean water (Abel, 1980). Sublethal levels of pollutants whilst not directly killing an organism may also be deleterious due to the effects on the reproduction

and physiology of the organism, as discussed, and also since behavioural changes may occur. The slow movements reported for cadmium exposed crayfish have also been observed, and a decrease in activity reported, for *Cambarus acuminatus* (Maciorowski *et. al.*, 1980). This could possibly lead to increased predation due to failure to escape. Insecticide induced activation and downstream drift has also been shown as a behavioural response of *Gammarus* spp. to both lethal and sublethal levels of Permethrin (Muirhead-Thompson, 1978), which also may prove deleterious, particularly in the case of a river such as the Leen, where the water quality deteriorates on moving downstream.

Note that this chapter has dealt specifically with the toxicity of cadmium and Lindane as separate entities under carefully controlled conditions. In the field it is likely that other potential pollutants may be present or that ideal conditions of high oxygen levels and moderate temperatures, for example, may not exist. Consequently due to effects of the multiple environmental parameters acting synergistically, the relative toxicities of each pollutant may be enhanced, and different results from those obtained above would be achieved (see 6).

TABLE 3.1 a AND b. RESULTS OF THE LD_{50} TRIALS USING LINDANE AS THE TOXICANT FOR a) JUVENILE, AND b) ADULT *A. PALLIPES*

In Tables 3.1 - 3.3 probit values shown in parentheses are those calculated for 0 or 100% using the corrected values derived after the method of Lichfield and Wilcoxon (1949). The results show the concentrations of toxicant used (in p.p.m.), the number of animals used at each test concentration (N), the observed percentage mortality after 96 hours (% M. 96 hrs) and its probit value. These results are used to calculate the LD_{50} 96 hours value and confidence limits shown in Table 3.4. the observed time elapsed until 50% mortalities occurred at each concentration are also shown in Tables 3.1 - 3.3 (LT_{50}, expressed in hours).

a) JUVENILES

CONCENTRATION (p.p.m.)	N	% M 96 hrs.	PROBIT.	TLM_{50} (hrs)
0, Control	20	0.0	-	10% mortality after 31 days
0.0005	15	0.0	(2.67)	240
0.0025	15	6.7	3.52	192
0.005	15	6.7	3.52	216
0.05	15	60.0	5.52	90
0.50	15	33.0	4.56	144
1.00	15	100.0	(6.28)	18
2.50	15	100.0	(6.34)	73
5.00	6	100.0	(6.48)	10
10.00	6	100.0	(6.64)	9

b) ADULTS

CONCENTRATION (p.p.m.)	N	% M 96 hrs.	PROBIT.	TLM_{50} (hrs)
0, Control	6	0.0	-	-
0.005	6	0.0	-	> 744
0.025	6	0.0	(3.25)	744
0.05	6	16.7	4.05	720
0.25	6	33.0	4.56	144
0.50	6	100.0	(5.03)	52

TABLE 3.2 a AND b. RESULTS OF THE LD_{50} TRIALS USING CADMIUM AS THE TOXICANT FOR a) JUVENILE, AND b) ADULT *A. PALLIPES*

a) JUVENILES

NOM. CONC.(p.p.m.)	ACT. CONC. (p.p.m.)	N	% M 96 hrs	PROBIT	TLM_{50}
0, Control	0.0	6	0.0	-	-
0.5	0.8 ±0.3	6	0.0	-	> 168
1.5	1.4 ±0.2	6	0.0	(2.95)	> 168
3.0	2.9 ±0.2	6	33.0	4.56	120
4.0	3.9 ±0.4	6	33.0	4.56	120
5.0	5.0 ±0.4	6	66.0	5.41	90
10.0	12.0 ±0.9	3	100.0	7.05	44
50.0	50.9 ±1.3	6	100.0	-	12

b) ADULTS

NOM. CONC.(p.p.m.)	ACT. CONC. (p.p.m.)	N	% M 96 hrs	PROBIT	TLM_{50}
0, Control	0	6	0.0	-	-
10.0	10.6 ±1.3	6	0.0	-	> 168
20.0	19.8 ±0.8	6	0.0	(3.12)	168
30.0	29.7 ±0.9	6	33.0	4.56	99
40.0	41.0 ±1.5	6	66.0	5.41	84
50.0	52.6 ±2.1	6	83.3	5.95	72

Nominal concentrations are the desired concentrations. The actual concentrations are those achieved during the first 96 hours (±1 S.E.), calculated as in 2.

TABLE 3.3 a AND b RESULTS OF THE LD_{50} TRIALS ON JUVENILE *A. PALLIPES* USING a) PERMETHRIN, AND b) MALATHION AS THE TOXICANTS.

a) PERMETHRIN

CONCENTRATION (p.p.m.)	N	% M 96 hrs	PROBIT	TLM_{50} (hrs)
0, Control	20	0.0	-	10% mortality after 31 days
0.0005	15	6.7	3.52	> 216
0.0025	15	20.0	4.16	> 216
0.005	15	13.3	3.87	176
0.05	15	46.7	4.92	88
0.50	15	86.7	6.13	41
1.00	15	100.0	(6.75)	21
2.50	15	100.0	(7.05)	15
5.00	6	100.0	(7.33)	15
10.0	6	100.0	(7.33)	15

b) MALATHION

CONCENTRATION (p.p.m.)	N	% M 96 hrs	PROBIT	TLM_{50} (hrs)
0, Control	20	0.0	-	-
0.0005	15	20.0	4.16	> 216
0.0025	15	53.3	5.08	56
0.005	15	13.3	3.87	170
0.05	15	6.7	3.52	184
0.50	15	20.0	4.16	168
1.00	15	93.3	6.48	42
2.50	15	93.3	6.48	24
5.00	6	100.0	(6.55)	24
10.00	6	100.0	(6.64)	24

TABLE 3.4 ANALYSIS OF TABLES 3.1 - 3.3 TO SHOW RESULTS OF CALCULATION OF LD_{50} 96 HRS (AND CONFIDENCE LIMITS), ALSO SHOWING REGRESSION DATA (CORRECTED TO TWO DECIMAL FIGURES), FOR CALCULATION OF LD_{50} 96 HRS BY THE METHOD OF LICHFIELD AND WILCOXON (1949)

TOXICANT	ADULT/JUV	EQUATION OF REGRESSION LINE	r	P	LD_{50} 96 HRS (p.p.m.)	95% CONFIDENCE LIMITS (p.p.m.)
LINDANE	JUVENILE	PROBIT. %M = 0.91. LOG.CONC. + 5.84	0.9427	<0.001	0.12	0.01 - 1.46
LINDANE	ADULT	PROBIT. %M = 1.23. LOG.CONC. + 5.39	0.9695	<0.05	0.48	0.08 - 3.07
CADMIUM	JUVENILE	PROBIT. %M = 4.34. LOG.CONC. + 2.32	0.9907	<0.001	4.14	2.45 - 7.00
CADMIUM	ADULT	PROBIT. %M = 6.69. LOG.CONC. - 5.44	0.9941	<0.01	36.45	25.92 -51.26
PERMETHRIN	JUVENILE	PROBIT. %M = 0.98. LOG.CONC. + 6.51	0.9877	<0.001	0.03	0.003 - 0.29
MALATHION	JUVENILE	PROBIT. %M = 0.62. LOG.CONC. + 5.75	0.7416	<0.02	0.06	0.002 - 2.44

TABLE 3.5 ANALYSIS OF TABLES 3.1 - 3.3 TO SHOW RESULTS OF CALCULATION OF LD_{50} 96 HRS VALUES FROM EXAMINATION OF TLM_{50} DATA ALSO SHOWING REGRESSION DATA (CORRECTED TO TWO DECIMAL FIGURES)

TOXICANT	ADULT/JUV.	EQUATION OF REGRESSION LINE	r	P	LD_{50} 96 HRS (p.p.m.)
LINDANE	JUVENILE	LOG. TLM_{50} = 1.53 - 0.31. LOG.CONC.	0.8408	<0.01	0.04
LINDANE	ADULT	LOG. TLM_{50} = 1.77 - 0.59. LOG.CONC.	0.8846	<0.05	0.44
CADMIUM	JUVENILE	LOG. TLM_{50} = 2.46 - 0.79. LOG.CONC.	0.9874	<0.001	3.98
CADMIUM	ADULT	LOG. TLM_{50} = 2.93 - 0.64. LOG.CONC.	0.9023	<0.05	30.36
PERMETHRIN	JUVENILE	LOG. TLM_{50} = 1.47 - 0.34. LOG.CONC.	0.9876	<0.001	0.03
MALATHION	JUVENILE	LOG. TLM_{50} = 1.67 - 0.20. LOG.CONC.	0.7404	<0.05	0.03

TABLE 3.6 A COMPARISON OF THE TOXICITY OF CADMIUM TO A VARIETY OF INSECT AND CRUSTACEAN ARTHROPODS

ORGANISM	AGE/SIZE	SUBLETHAL CONC. (p.p.m.)	LD_{50} 96 HRS (p.p.m.)	NOTES	REFERENCE
FRESHWATER					
Daphnia galeata mendotae	-		0.03		Marshal, 1979
Daphnia magna	-	0.0007	0.005	Time scale of LD_{50} not given	Piotrowski and Coleman, 1980
Orconectes propinquus	Adult	0.01 - 1.0		No mortalities up to 190 hrs.	Gillespie *et.al.*, 1977
Cambarus latimanus	Adult	0.005 - 0.1		2 died after 14 days at 0.01 p.p.m.	Thorpe *et. al.*, 1979
Austropotamobius pallipes	5.6 ±0.4mm		3.98 - 4.14	Carapace length measurements, hatchlings	Author
A. pallipes	35.4 ±1.1mm		30.36 - 36.45	Carapace length measurements, adults	Author
Ephemerella subvaria	-		2.0		in, Club *et. al.*, 1975
E. grandis grandis	-		28.0		Club *et. al.*, 1975
Pteronarcella badia	-		18.0		Club *et. al.*, 1975
MARINE					
Uca pugilator	Adult		6.8 - 46.6	LD_{50} dependent upon salinity and temp.	Vernberg *et. al.*, 1974
Eurypanopeus depressus	Adult		4.9	72 hr. LD_{50}	Calabrese *et. al.*, 1977
Paratya tasmaniensis	-		0.06		Thorpe and Lake, 1974
Palaemonetes vulgaris	-	0.075			Piotrowski and Coleman, 1980
Carcinus maenas	-	0.5			Piotrowski and Coleman, 1980
Mysidopsis bahia	-	0.0106			Piotrowski and Coleman, 1980
Palaemonetes vulgaris	Adult		0.76		Nimmo *et. al.*, 1977
Penaeus duorarum	Adult		3.50		Nimmo *et. al.*, 1977

TABLE 3.7 A COMPARISON OF THE TOXICITIES OF BHC(HCH) ISOMERS TO DIFFERENT CRUSTACEANS

ORGANISM	AGE/SIZE	CHEMICAL	LD_{50} 96 HRS (p.p.m.)	NOTES	REFERENCE
FRESHWATER					
Daphnia pulex	-	LINDANE	0.46		in, US.EPA-440, 1980
Daphnia magna	-	LINDANE	0.49	LD_{50} 48 hrs.	Macek *et. al.*, 1976
Daphnia magna	1 day	α HCH	0.80	LD_{50} 48 hrs.	Canton *et. al.*, 1975
Gammarus fasciatus	-	LINDANE	0.01		Sanders, 1972
Gammarus lacustris	2 months	LINDANE	0.48		Sanders, 1969
Assellus brevicaudus	-	LINDANE	0.01		Sanders, 1972
Astacus leptodactylus	80 - 90mm	LINDANE	0.04	TOTAL LENGTH	Airaksinen *et. al.*,1976
Austropotamobius pallipes	5.2 ±0.3mm	LINDANE	0.04 - 0.12	CARAPACE LENGTH	Author
A. pallipes	28.1 ±1.8mm	LINDANE	0.44 - 0.48	CARAPACE LENGTH	Author
MARINE					
Penaeus duorarum	-	LINDANE	0.00017	ASSUME ADULT	Schimmel *et. al.*, 1977a
P. duorarum	-	TECHNICAL BHC	0.00034	ASSUME ADULT	Schimmel *et. al.*, 1977a
Palaemonetes pagio	-	LINDANE	0.00444	ASSUME ADULT	Schimmel *et. al.*, 1977a
Penaeus vulgaris	-	LINDANE	0.01		in, US.EPA-440, 1980
Crangon spp.	-	LINDANE	0.005		in, US. EPA-440, 1980

TABLE 3.8 A COMPARISON OF THE TOXICITIES OF THE INSECTICIDES LINDANE, MALATHION AND PERMETHRIN TO A VARIETY OF FRESHWATER CRUSTACEANS

ORGANISM	AGE/SIZE	LINDANE	LD_{50} 96 HRS.(p.p.m.) PERMETHRIN	MALATHION	NOTES	REFERENCE
Gammarus fasciatus	-	0.010	0.011	0.00076	Natural pyrethrun used	Sanders, 1972
Gammarus	-		0.001		LC_{90-95} 24 hrs.	Muirhead-Thomson,1978
G. lacustris	-	0.48	0.00028		LD_{50} 24 hrs.	Sanders, 1969
Daphnia magna	-	1.25		0.0009		Frear and Boyd, 1967
Palaemonetes kadiakensis	-			0.09		Sanders, 1972
A. pallipes	5.2±0.3mm	0.04-0.12	0.03	0.03-0.06	Carapace length	Author
Procambarus clarkii	4-10g			(20)	No mortalities	Muncy and Oliver,1963
P. clarkii	8-12mm				Newly hatched	Jolly *et. al.*, 1978
P. clarkii	20-30mm				Juvenile	Jolly *et. al.*, 1978
Procambarus acutus acutus	Juvenile			50		Cheah *et. al.*, 1976
Orconectes naiis	Adult			(100)	No mortalities	Sanders, 1972
O. naiis	Juvenile			0.18		Sanders, 1972

FIG. 3.1 TO SHOW THE RELATIONSHIP BETWEEN TLM_{50} AND TOXICANT CONCENTRATION FOR ADULT (—) AND JUVENILE (---) *A. PALLIPES* EXPOSED TO LINDANE

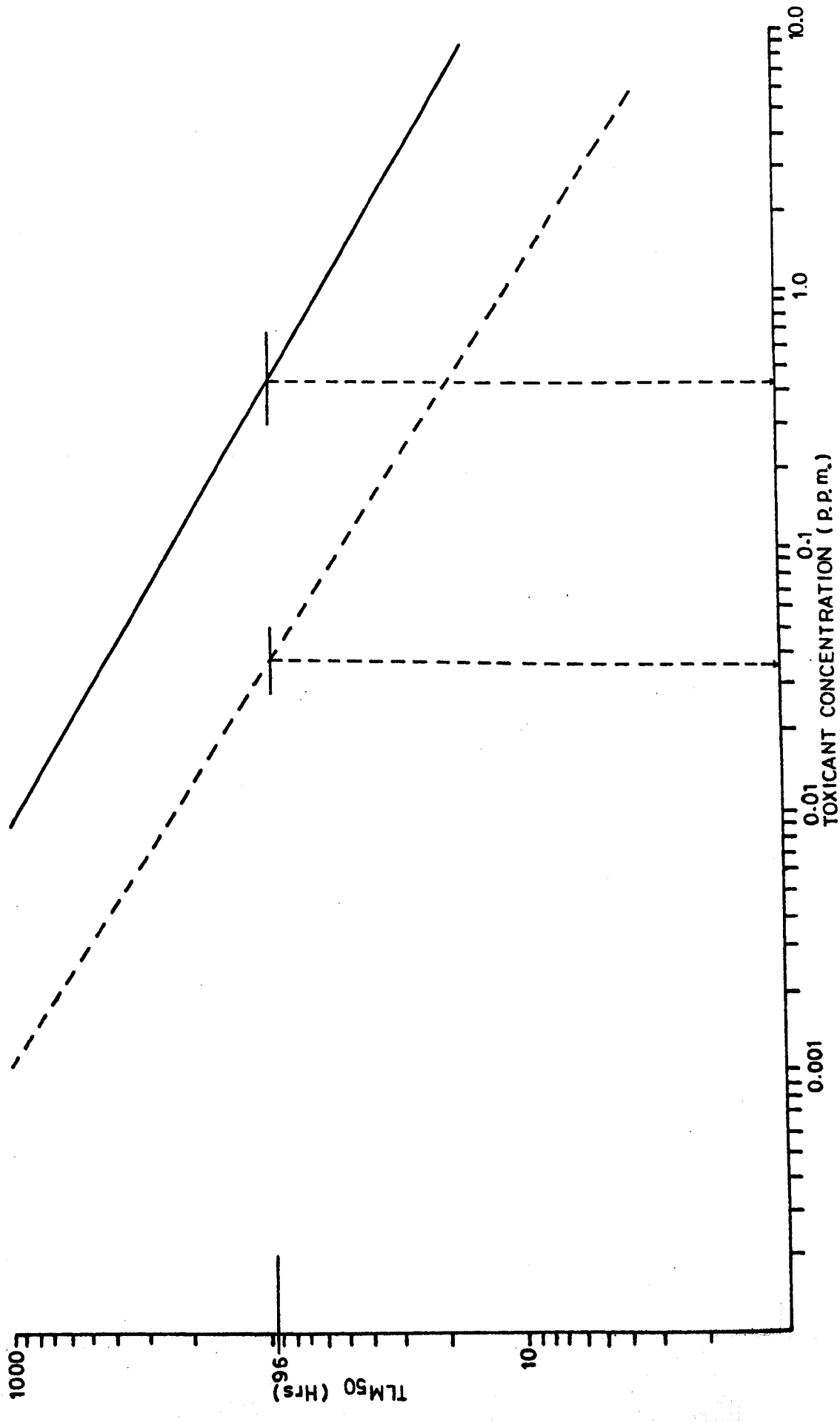


FIG. 3.2 TO SHOW THE RELATIONSHIPBETWEEN TLM_{50} AND TOXICANT CONCENTRATION FOR ADULT (——) AND JUVENILE (- - -) *A. PALLIPES* EXPOSED TO CADMIUM

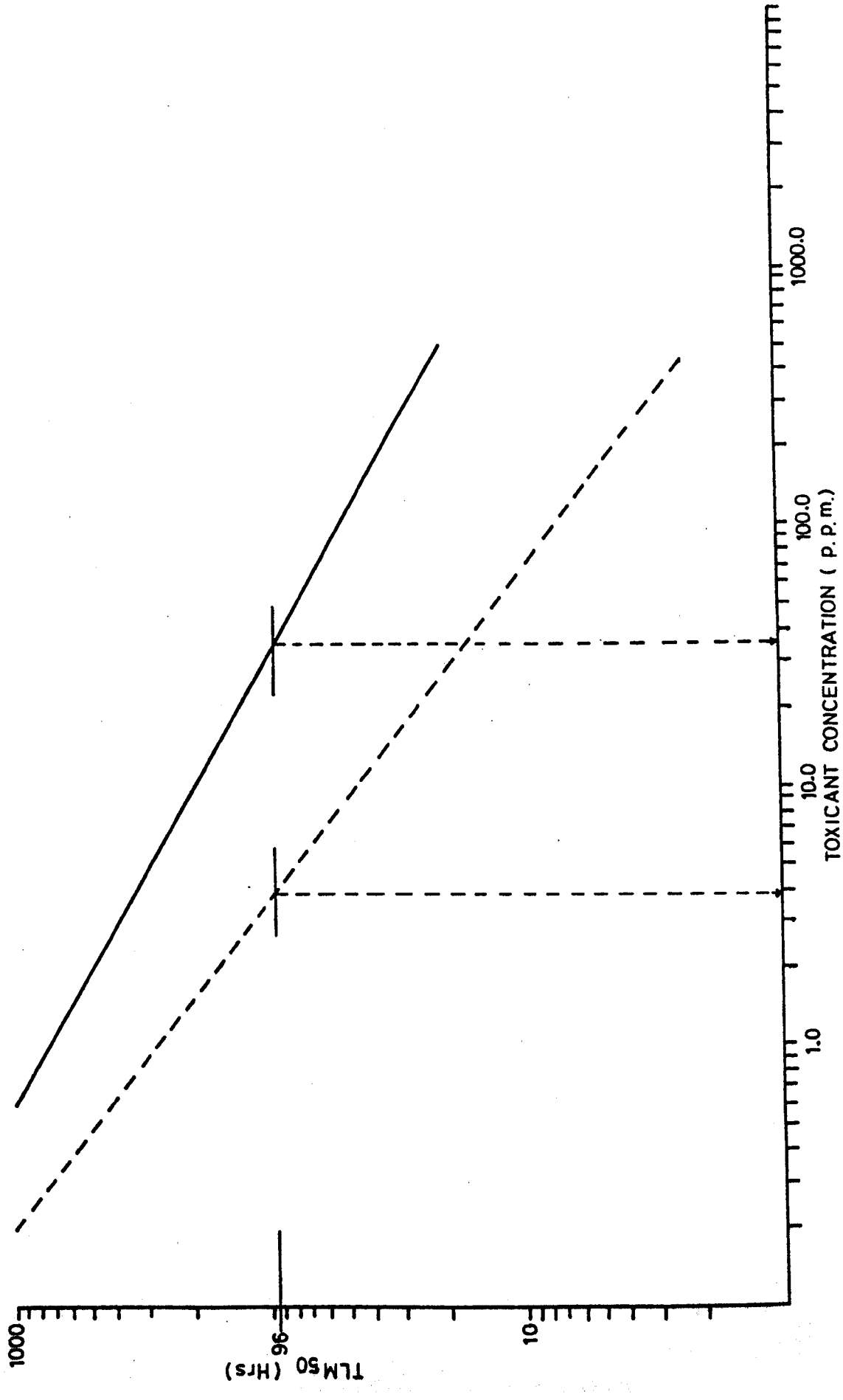
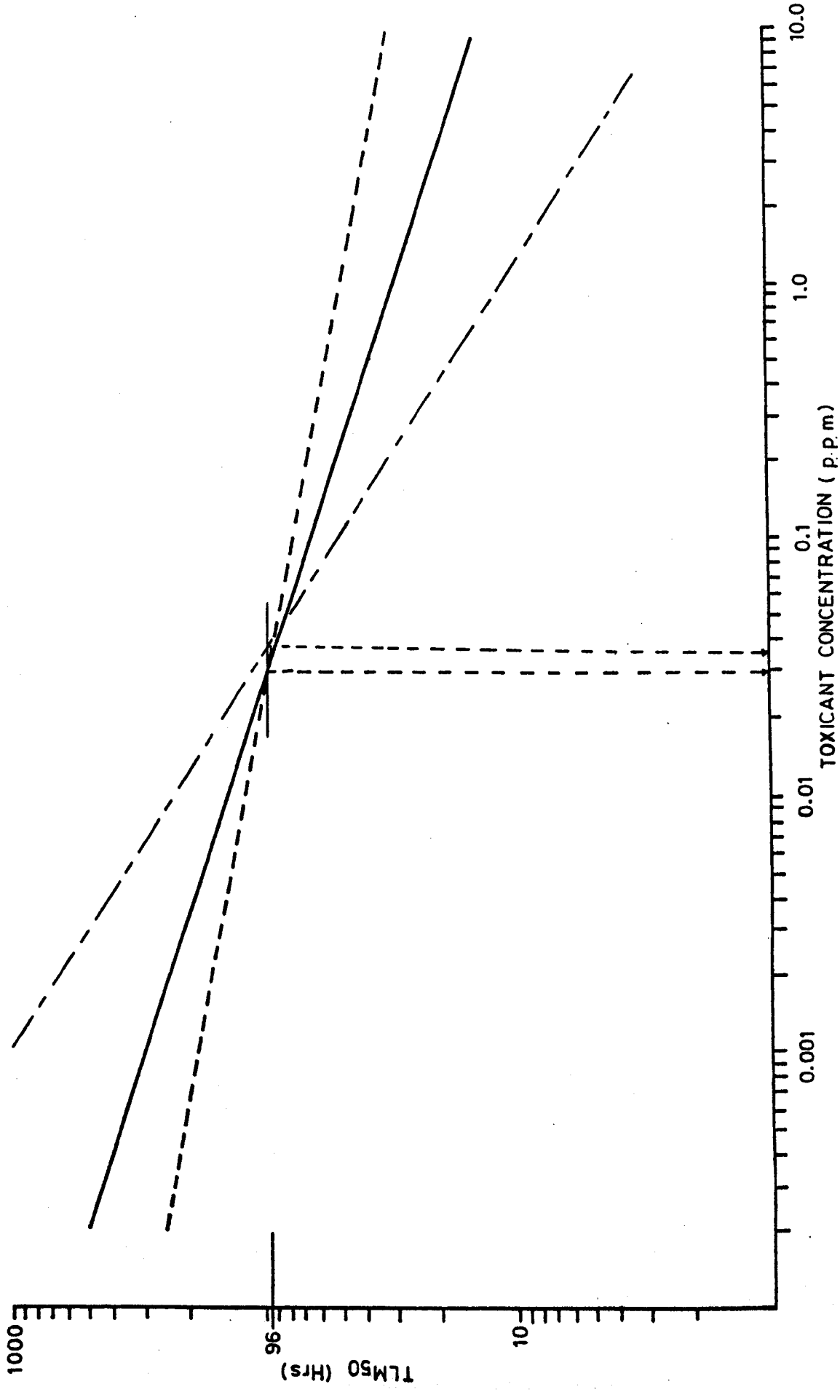


FIG. 3.3 TO SHOW THE RELATIONSHIP BETWEEN TLM_{50} AND TOXICANT CONCENTRATION FOR JUVENILE *A. PALLIPES* EXPOSED TO PERMETHRIN (——) MALATHION (---) AND FOR COMPARISON, LINDANE (— - —)



CHAPTER 4

THE ACCUMULATION OF CADMIUM AND LINDANE IN THE TISSUES OF *A. PALLIPES*

4. INTRODUCTION

The toxicity of cadmium and Lindane to *A. pallipes* has been established, and the mechanism of toxicity has been described (3). Loss of osmoregulatory control and respiratory function at the gills has been proposed as a likely cause of mortalities, and this chapter aims to establish whether the toxicants studied are accumulated in the gills, or indeed any other tissues. Furthermore this study is essential if crayfish are to become a food resource since, if accumulation occurs, *A. pallipes* could potentially become a vector for the transmission of toxic substances to man. In addition to the uptake of toxicant, depuration in clean water is studied. Such a situation may occur in the field if a sudden unsustained flush of pollutant occurred, and these studies will help to establish if *A. pallipes* would be able to survive such conditions.

A. pallipes is relatively tolerant to cadmium compared with Lindane (see 3). Thus, the concentrations of cadmium that it was possible to use in uptake studies were well within the sensitivity of the equipment available. Atomic absorption spectrophotometry was used, and concentrations as low as 0.01 p.p.m. could be detected. Hence it was possible to run a large number of experiments in order to establish the dynamics of cadmium accumulation and elimination by *A. pallipes*, and naturally occurring levels of heavy metals were also monitored.

In order to detect Lindane, gas-liquid-chromatography (G.L.C.) techniques are required, and with standard equipment a general

screen is only possible to levels of 1 p.p.m. In order to detect lower concentrations an electron-capture detector is also required. Furthermore, a highly specific extraction procedure must be devised in order to remove Lindane from both water, and crayfish tissues. Since only low concentrations of Lindane were to be studied (~0.03 p.p.m.) it was therefore decided that the use of radiolabelled Lindane would be the most effective method of establishing the dynamics of accumulation of this toxicant. Unfortunately this has meant that no estimates of the presence of insecticides from natural populations was possible in this study, although American studies have established that accumulation of organochlorine compounds such as D.D.T. does occur in natural populations of crustaceans (see 4.2(iii)).

4.1 CADMIUM ACCUMULATION IN TISSUES OF *A. PALLIPES*

4.1(i) MATERIALS AND METHODS

In order to establish the dynamics of cadmium accumulation in the different tissues of *A. pallipes* a series of experiments was conducted. All experiments were conducted at 10°C ± 1°C, except those using gill tissue obtained after the respirometry studies (see 5), which were conducted at 15°C ± 1°C. All animals were subject to the usual pre-experimental procedure (see 2) and a mixture of intermoult males and females from both Nanpantan Reservoir and Markfield Quarry were used as detailed in the individual experimental conditions. The pH of the water was monitored regularly, and was not observed to go beyond the range 7.10 - 7.85 at any time. The experiments conducted were:

1. Cadmium uptake by male and female crayfish.

Markfield Quarry stock were used, and three animals of each sex (males - C.L. 35.5 ± 2.6 mm; females - C.L. 29.3 ± 0.8 mm) were exposed to a nominal concentration of 10 p.p.m. cadmium for 7 days. The following tissues were then examined for their cadmium content: cuticle, gills, hepatopancreas, alimentary tract, gonads, and tail muscle. The concentrations of cadmium found in each tissue of each sex were compared by means of the t-test.

2. Cadmium elimination by crayfish.

Intermoult males of the Markfield Quarry stock (C.L. 33.0 ± 1.9mm) were used. 15 animals were exposed to a nominal concentration of 10 p.p.m. cadmium for 7 days after which 3 crayfish were removed and all the tissues described previously examined for their cadmium content. The remaining 12 crayfish were returned to clean water, which was changed twice weekly, and after each of 7, 14, 21,

and 28 days 3 animals were removed and their tissues analysed. One way analysis of variance was employed to determine whether any significant reduction in the tissue concentration of cadmium occurred with increasing time in clean water.

3. Uptake of cadmium by the gills of crayfish exposed to toxicant for an increasing time period.

Gills of crayfish from Markfield Quarry stock were employed after being used for the respirometry trials, as described in Chapter 5. Other authors have also analysed gill tissues after use in oxygen uptake experiments (e.g. Anderson, 1978; Engel, 1979) and it is not considered likely that loss of cadmium from the tissue would have occurred during those experiments, since the cadmium is likely to be bound to proteins, possibly in cell membranes and mitochondria (see 3(iv)). 15 animals of both sexes were exposed to nominal concentrations of 1 p.p.m. and 10 p.p.m. cadmium for up to 9 days, 3 animals being removed on each of days 1, 3, 6, and 9 for analysis. The cadmium concentration in the gills increased linearly with time, so regression analysis was used to test the significance of the results.

4. Uptake of cadmium by the gills of crayfish exposed to a range of toxicant concentrations for a period of 3 days.

This experiment also employed gills of crayfish from Markfield Quarry stock previously used in respirometry experiments, and described in Chapter 5. Six sets of gills taken from 3 animals exposed to each of 1 p.p.m., 5 p.p.m., 10 p.p.m. and 50 p.p.m. nominal cadmium concentrations for 3 days were analysed. Two sets of gills only were analysed from one crayfish exposed to 25 p.p.m. cadmium because of mortalities at this concentration.

Mortalities had also occurred at 50 p.p.m. but sufficient animals remained to provide a full gill sample. Linear regression analysis was applied to the results.

5. Uptake of cadmium over an extended time period by crayfish exposed to a range of toxicant concentrations extending from very low sublethal levels to relatively high levels.

This experiment was not designed specifically to monitor cadmium uptake, and was a spin off from toxicity tests described in Chapter 3. Nanpantan animals, which proved to be less sensitive to cadmium than Markfield animals, but which had been exposed to low concentrations of cadmium for toxicity trials and had survived were used (as described, see 3(ii)). Six crayfish were exposed to each of concentrations of 0.25 p.p.m., 0.5 p.p.m., 2.5 p.p.m., 5.0 p.p.m., 10.0 p.p.m., 20.0 p.p.m. and 30.0 p.p.m. for 4 weeks. Water and toxicant was changed daily for the first 4 days, and weekly thereafter. All of the tissues detailed above were analysed for the cadmium content in one crayfish at each concentration each week for 4 weeks. The experiment was not continued to 6 weeks due to mortalities. Owing to the fact that only one animal was analysed at each concentration the results bear no statistical significance, but are nevertheless presented since they do show a positive trend towards increased tissue cadmium accumulation with increasing time.

6. The removal of cadmium from the water in which the crayfish were placed.

Collier *et. al.*, (1973) state that cadmium is very stable in water and levels are not reduced by aeration. They quote a study in which the cadmium concentrations had fallen by only 5% in

a period of 264 hours (11 days, range 0.1 - 400 p.p.m.). It was decided to monitor the removal of cadmium from the water daily during the second week of experiment 5, when 5 crayfish were in 5 litres of water of concentrations 0.25 - 30 p.p.m. Aquaria containing 5 litres of water and 0 p.p.m. and 10 p.p.m. cadmium ions but no crayfish were also monitored. Comparison between the two 10 p.p.m. aquaria (± crayfish) was effected using the t-test.

7. Controls

Controls for the trials with Nanpantan stock employed 3 control Nanpantan animals from toxicity tests. All of the tissues examined from treated animals were subject to analysis, and cadmium was the only metal monitored. Six Markfield Quarry animals not exposed to toxicant were analysed for their cadmium content and acted as the controls for the trials employing Markfield stock. In order to establish whether any other metals were accumulated in the tissues from the natural environment (Markfield Quarry), copper, nickel, lead, zinc, iron, and manganese were also assayed within each tissue. A single sample of Markfield Quarry water was also analysed for all of these metals.

An additional set of data was generated for crayfish taken from the River Leen population study area (see Part I, 1.1 and 3.3(i)). The tissues of 6 animals were analysed for all of the above metals, and in addition a single sample of water taken from both the population study areas, and the Leen below the confluence of the East and West branches, was analysed. Nottingham University tap water, taken from the stock tank (see 2) was analysed in experiment 6, for cadmium only, and is the control

for experimental-water cadmium content.

EXPERIMENTAL PROCEDURE

After exposure to cadmium for the required time, crayfish were flushed with clean water to remove external cadmium, placed into labelled plastic bottles, and deep frozen in order to kill them, and also to prevent microbiological spoilage and autolytic decay of the tissues (e.g. see Mees, 1983). They were kept frozen until such time as it was convenient to analyse them. They were then thawed out at ambient temperatures, and the tissues (cuticle, gills, hepatopancreas, alimentary tract, gonads, tail muscle) dissected out into preweighed and numbered 10 ml soda vials and dried as in 2, in order to obtain the dry weight of tissue analysed. The vials had all previously been washed in 10% v:v nitric acid and rinsed with deionized water in order to remove any metals adhering to the glass.

The method employed to monitor cadmium levels in water samples and tissues was adapted from one described by the Department of the Environment (D.o.E., 1976). Water samples were acidified with a few drops of concentrated hydrochloric acid, and stored frozen in a polyethylene bottle until analysis. Acidification maintains the metal in ionized form and keeps it in solution, minimizing adsorption onto the walls of the container.

The tissue analysis was more complex. Concentrated nitric acid, hydrochloric acid, and perchloric acid were mixed in a 1 litre measuring cylinder (in a fume cupboard) in the ratio of 4:1:1 respectively. Approximately 8 mls of this mixture was added to each vial containing the dried tissue, and they were then left overnight to digest. Not all of the tissues would be dissolved

overnight, particularly the cuticle and gut of the alimentary tract, but complete tissue digestion occurred at the next stage. The liquid digest and any remaining tissue were tipped into 50 ml conical flasks (prerinsed with 10% HNO_3) and placed on a sand bath (heated to 80°C - 100°C) in a fume cupboard. The acid started to boil and evapourate off, and was allowed to do so until the liquid had become colourless and only 0.5 - 1.0 mls remained. At this point the flasks were removed and allowed to cool. The precise point at which to remove a flask may be determined when it is seen to fill with white clouds of gas from the acid. When cool, a small amount of double distilled and deionized water was added to the flask from a wash bottle, rinsed around, and then poured through a funnel into a 25 ml volumetric flask. The conical flask and funnel were rinsed again, and the volume made up to precisely 25 ml. The final sample was then tipped into a labelled polyethylene bottle and stored frozen until required for analysis.

Analysis of all samples was conducted on the Nottingham University Botany Department atomic absorption spectrophotometer (A.A.S.). The equipment is calibrated using double distilled deionized water to produce a zero reading, and a 5 p.p.m. standard made up from a BDH cadmium chloride solution prepared specifically for A.A.S. to give a 5 p.p.m. reading. After calibration the samples may be put directly through the A.A.S. and the amount of cadmium read from the L.E.D. display as parts per million (p.p.m.). Any sample containing a concentration higher than 5 p.p.m. was suitably diluted and the reading taken again. A corresponding multiplication factor was then applied to the results.

In order to establish that the cadmium content indicated for each tissue actually came from the tissues and was not a result of the experimental procedure, 5 blanks were also put through exactly the same routine i.e. on each occasion, 5 vials containing no tissue had acid added and were taken through to the stage of analysis. If any cadmium was detectable then the mean value of the 5 blocks was determined and subtracted from each sample value obtained. Generally it was around 0.04 p.p.m.

Next, in order to determine that all of the cadmium in the tissues was being extracted and detected by the above procedure, percentage recovery trials were conducted using known quantities of metal. Five untreated animals had their tissues dissected out and dried. A 125 p.p.m. stock solution (nominal concentration) was then prepared by dissolving 0.2528g of cadmium chloride in 1 litre of deionized water (calculated concentration 124.43 p.p.m.). 1 ml of this stock solution in 24 mls of deionized water produces a calculated concentration of 4.98 p.p.m. Five samples of 1 ml stock:24 ml deionized water were prepared to check the accuracy of this calculation on the A.A.S., and analysed at 5.06 ± 0.06 p.p.m.

1 ml of 125 p.p.m. stock was added to each of the dissected tissues of the 5 animals, and the tissues were allowed to rehydrate. They were then redried and the described experimental procedure was applied. The resulting 25 ml of sample solution would thus be expected to produce a result of 4.98 p.p.m. by calculation, and 5.06 ±0.06 p.p.m. by analysis. The amounts recovered, however, tended to be marginally less than this, and were calculated as a percentage of that expected (by analysis). The proportions

were; Cuticle (91.3 ± 2.4%), gill (88.6 ± 3.3%), hepatopancreas (90.7 ±2.5%), alimentary tract (90.7 ±3.0%), gonads (90.6 ±1.8%) and tail muscle (91.3 ± 2.4%). Consequently all results obtained during the uptake trials were first corrected for the blank, and then a percentage recovery was applied to indicate the actual amounts of cadmium in the tissues. Thus the results were divided by the following factors; Cuticle (0.913), gill (0.886), hepatopancreas (0.907), alimentary tract (0.907), gonads (0.906), tail muscle (0.913).

The results obained in this way are expressed as p.p.m. cadmium ions in solution. The final results, however, are expressed as mg. of cadmium per g. dry tissue. This is calculated in the following manner:

$$1 \text{ p.p.m.} \equiv 1 \text{ mgl}^{-1}$$

$$\therefore x \text{ p.p.m. in 25 mls} \equiv x\text{mgl}^{-1} \times \frac{1}{40} \text{ l}$$

$$= \frac{x}{40} \text{ mg of cadmium}$$

All this is from the tissues, and so dividing by the dry weight (tg.) gives the result as mg Cd^{2+} g^{-1} dry weight of tissue.

$$\text{i.e. } \frac{x}{40} \text{ mg} \div \text{tg} = \text{mgg}^{-1}$$

4.1(ii) RESULTS

The results of experiments 1 - 7 are expressed in Tables 4.1 - 4.7 respectively. It is pertinent, however, to present the results of the control experiment first, Table 4.7, and also that of the removal of cadmium ions from solution, Table 4.6. Neither Nottingham tap water, nor water from either of the population

study areas contained detectable amounts of cadmium. Thus animals had not previously been environmentally exposed to cadmium (see also 1, 3(iv)), and neither were they exposed to cadmium whilst in the stock tanks prior to experimental use. The presence of other metals in the environment is reported in Table 4.7, and it will be seen that in the study areas the concentrations of all metals are very low except for iron. In the River Leen after the confluence of the two branches the levels of all metals are appreciably higher and this is explained by the mine workings along the West branch. Zinc, cadmium and iron especially are higher than in the study area, although the level of cadmium is suspiciously high and may indicate some contamination of the sample. However, zinc and cadmium are closely related and usually occur together, and so the fact that the level of zinc is also elevated does tend to support the findings for cadmium. Also cadmium is reported to be found in mining effluents (see 1), though not in such high concentrations. Thus it appears that crayfish from the Leen population could be environmentally exposed to cadmium if they were to move downstream, although they were rarely found below the confluence (see Part I, 3.3).

Examination of all the tissues of crayfish from each of the Nanpantan, Leen, and Markfield populations revealed that cadmium was not present in detectable amounts. Thus in experiments 1 - 5, any cadmium detected derives from that added to the water by the author. Certain other metals, detailed in Table 4.7, were detected in the tissues of River Leen and Markfield Quarry animals. In general the highest concentration occurred in either the gills (Leen: zinc, copper, nickel, lead. Markfield: zinc, copper, iron,

nickel, lead), or the gut (Leen: manganese, iron. Markfield: manganese), presumably indicating that the source of accumulation was either from the water or food. Only iron differs between the populations, and the actual levels of iron in the gills and gut for each population are similar. The levels of the metals in the other tissues are very low, although appreciable amounts of zinc, copper, and iron occur in the hepatopancreas of Leen animals, and of zinc, manganese, copper and iron in the hepatopancreas of Markfield animals. The overall concentrations of each metal are very similar in both Leen and Markfield animals, and indicate that Leen animals need not necessarily have moved beyond the confluence in order to accumulate metal ions.

Experiment 6 revealed that at most concentrations of cadmium, approximately 85% of the initial level remained in solution at the end of a period of 7 days. This reduction in the amount of available cadmium was not due to uptake by the crayfish in the tanks since a similar reduction in concentration occurred for a tank at 10 p.p.m. containing no animals. There was no significant difference in the change of concentrations in this tank or one at 10 p.p.m. containing 5 animals ($t = 0.01$, $P > 0.1$). This indicates that the amount of cadmium taken up by the tissues is insignificant compared with the amount coming out of solution either by precipitation or by adsorption onto the surfaces of the aquaria. Thus it was not possible accurately to monitor removal of cadmium from the environment by crayfish, and experiments 1 - 5 which measured uptake by the tissues were the more sensitive tests.

Experiment 1 demonstrated that no sexual differences occurred for the uptake of cadmium into any of the tissues examined

($P > 0.10$ in each case, Table 4.1). The highest metal concentrations were seen to exist in the gills, followed by the cuticle and hepatopancreas, although the amount in the gills was of an order of magnitude greater. Only very small amounts of cadmium were found in the alimentary tract, gonads and tail muscle.

The results of experiment 2 presented in Table 4.2, are illustrated in Fig. 4.1. If the cadmium contents of the gills and of the total body burden are examined, it will be seen that a general decrease in the level of cadmium was observed, but this was not consistent due to an unusually low result for Day 14, and there was no statistically significant trend with increasing time in clean water ($P > 0.05$, Table 4.2). Of the other tissues, the level of cadmium in both the cuticle and alimentary tract was initially seen to decrease from day 0 to day 7, and thereafter to increase with increasing time in clean water. This increase proved to be significant at the 5% level for the cuticle, and the 1% level for the alimentary tract. An increase in the level of cadmium in the hepatopancreas was also observed, and was significant ($P < 0.01$). It increased consistently from day 0 to day 28. Only barely detectable amounts of cadmium occurred in the gonads and tail muscle. That in the gonads was eliminated by day 7, whilst no cadmium was detected in the muscle tissue until day 21.

The gills appear to be the chief site of uptake of cadmium. Experiments 3 and 4 (Tables 4.3a, 4.3b, 4.4 and Figs. 4.2 and 4.3) examine the uptake of cadmium by gills of crayfish exposed in the first experiment to 1 p.p.m. and 10 p.p.m. cadmium for up to 9 days, and in the second to increasing cadmium concentrations

for 3 days. A positive linear relationship exists for uptake of cadmium with time at both concentrations studied, and this relationship is significant in both cases at the 1% level (Table 4.3b). A positive linear relationship, significant at the 1% level, is also apparent with increasing external cadmium ion concentrations leading to increasing internal tissue concentrations.

Experiment 5 was not designed specifically as an 'uptake experiment' and is statistically insignificant since only one animal was employed on each occasion. However, the results as presented in Table 4.5 do support the results of the previous experiments. Cadmium only occurred consistently in the gills and cuticle, but what is significant is that it was accumulated even when the external cadmium concentration was as low as 0.25 p.p.m. There were appreciable amounts of cadmium in the hepatopancreas only at the highest external concentrations, and it was more prevalent at all concentrations as the time of exposure to toxicant increased. A similar pattern emerges for the alimentary tract and also the gonads and tail muscle, the latter two tissues, as previously, containing the smallest quantities of metal. Only the results for cadmium accumulation by the gills have been illustrated, showing that increasing cadmium uptake occurred with both time of exposure and increasing external cadmium concentration, in agreement with experiments 3 and 4 (see Fig. 4.4). The results for 10 p.p.m. from experiment 3 are included in Fig. 4.4 and show that uptake rates by both Nanpantan stock used in experiment 5, and Markfield stock used in experiment 3, are very similar. It is also apparent that considerably higher accumulation of cadmium may occur with prolonged exposure than

over the 7 and 9 day periods previously studied.

4.1(iii) DISCUSSION

The results expressed above showed that crayfish in the Midlands populations studied were not environmentally exposed to cadmium and did not normally have cadmium in the tissues, although certain other metals may be present in small quantities. When cadmium was detected, its source derived from that added to the water during the course of the experiments. It was found that cadmium accumulates in the tissues, most being located in the gills, followed by the cuticle, hepatopancreas, alimentary tract, and gonads and muscle, in descending order. No differences were observed in the levels of accumulation of males and females. A positive correlation was shown to exist, with internal cadmium tissue concentrations increasing with both (i) increasing time of exposure to cadmium for any given external concentration, and (ii) with increasing external cadmium ion concentrations for a given time of exposure. Previously exposed crayfish put into clean water were found to be able to translocate the cadmium from their gills to other tissues, and some removal of cadmium from the body appears to occur with a general reduction in the total body burden being observed with time. The observation contrary to that of Collier *et. al.*, (1973) that cadmium ions in solution decrease with time, even in the absence of animals, reinforces the policy suggested in Chapter 2 that both water and toxicant should be changed regularly during toxicology studies in order to maintain the concentrations of toxicant at the desired levels.

A vast literature exists on the uptake and accumulation

of heavy metals by aquatic organisms, and this is the case even when the field is restricted to cadmium and crustaceans. A large proportion of the previous studies have been directed towards marine decapod crustaceans, but literature also exists on freshwater Crustacea, including a study of *A. pallipes* which reported natural levels of copper found in various tissues, and examined the metabolism of low level doses of radiolabelled zinc (Bryan, 1967). Wright (1978) has reviewed the literature concerning heavy metal accumulation by aquatic invertebrates, and includes brief sections on cadmium and Crustacea, whilst Fowler *et. al.*,(1981) have reviewed the cellular mechanisms of trace metal uptake (see also Caprene and George, 1981). A brief review of the uptake and excretion of cadmium by marine organisms exposed to low levels of cadmium in sea water has also been provided by McLeese (1981).

The first result reported in this study related to the levels of various heavy metals found to occur naturally in *A. pallipes*. Table 4.8 compares these findings with those for other freshwater crayfish, whilst Ray *et. al.* (1979a) have reported the levels of various metals found in the marine shrimp *Crangon septimosa*. These are considerably lower than those found in the freshwater crustaceans reported (Metal, in $\mu g.g^{-1}$ dry weight: Cd, 0.4 - 1.45. Cu, 7.5 - 11.5. Pb, 1.5 - 8.0, Zn, 1.7 - 15). This may either be the result of different levels of environmental exposure, or possibly because marine crustaceans have a greater ability to eliminate heavy metals from their tissues. A comparison of the freshwater crayfish species shows that in each case cadmium is the least evident metal, and thus indicates that naturally occurring environmental levels are also low. The other metals

are present in *A. pallipes* in roughly equal amounts (this study), whilst lead is apparently less abundant than copper or zinc for crayfish in the Fox River (Anderson, 1977). The apparently higher levels of metals found in this study are explained by the fact that the results relate to specific tissues which concentrate the metals, whilst those of the study by Anderson (1977) average out the total body metal content. Overall the metals are present in similar orders of magnitude and in similar proportions. All of the results which relate to *A. pallipes* give the levels of metals in both hepatopancreas and gill tissue. For both copper and zinc Bryan (1967) found that high levels of metal occurred in the hepatopancreas whilst those in the gill were much lower. The levels in the hepatopancreas were of a similar order to those reported in the gills for copper, and the hepatopancreas for zinc (this study) in Midlands populations. However, this study reported higher levels of these metals in the gills than the hepatopancreas, the reverse of the situation shown by Bryan (1967). Tolba (unpubl.) similarly reported higher levels of zinc (also Pb, see Table 4.8) in the gills, but the levels of copper were highest in the hepatopancreas. The differences reported between this study and that of Bryan (1967) are thus substantiated for Midlands populations. Note, however, that a possibility of some experimental error exists in this study for all metals other than cadmium, since blank and percentage recovery trials were only conducted with cadmium. Thus any slight errors in the concentrations of metals detected are amplified when the result is translated into metal content per unit dry weight, due to the very small quantities of dry tissue analysed. That the results

prove to be of a similar order to those of other authors, however, supports the validity of the overall findings.

Table 4.9 summarizes part of the literature which deals with the uptake of cadmium by Crustacea. Cadmium uptake may occur from the sediment in which an animal lives (e.g. Ray *et.al*, 1980a; Ray *et. al.*, 1981b), from the food it eats (e.g. Nimmo *et. al.*, 1977), or from solution in the aquatic environment (this study). However, Macek *et. al.*, (1979) working with the blue gill sunfish concluded that cadmium accumulation in the tissues from the diet was insignificant compared to accumulation from an aqueous source. Similarly in crustaceans it was found that about 15,000 times more cadmium was accumulated from water, compared with equivalent concentrations in food (Nimmo *et. al.*, 1977). Most studies examine the uptake of cadmium from an aqueous source.

Temperature has been shown to affect the rate of uptake of cadmium, and for marine crustaceans salinity is also important. Low salinity and high temperatures tend to cause the accumulation of cadmium (e.g. Hutcheson, 1972). Vernberg *et. al.*,(1974), however, found that the total body burden of cadmium was similar whatever regime of temperature and salinity was employed, but that these parameters affected the site of accumulation, and that the relative amounts of cadmium in either the gills or hepatopancreas were dependent upon the regime employed. The effect of temperature on *A. pallipes* was not studied since most experiments were conducted at 10°C ± 1°C. However, the rate of uptake of cadmium by gills of crayfish from the Nanpantan stock, maintained at 10°C, was seen to be similar to that of gills of crayfish from Markfield stock, maintained at 15°C (Fig. 4.4). This may indicate no

substantial temperature differences, but it must be noted that the result with the Nanpantan stock was statistically not significant, so no sound judgements can be made.

The rate of cadmium accumulation was shown to be directly proportional to the external concentration of cadmium, and also to the time of exposure to cadmium (this study). A positive linear relationship was shown to exist in each case. Similar results have been obtained for other crustaceans (e.g. *Crangon crangon* - Delthlefsen, 1977; *Penaeus duorarum* - Nimmo *et. al.* , 1977; *Homarus americanus* - Thurberg *et. al.*, 1977; *Gammarus pseudolimnaeus* - Spehar *et. al.*1978). For another crayfish, *Orconectes propinquus*, the uptake of radioactive cadmium - 109 has been studied at very low levels of exposure (Gillespie *et. al.*, 1976). A significant increase in the accumulation of cadmium - 109 was reported with time, but there was no significant difference between the rates of uptake of crayfish exposed to the lowest concentrations (0.01 and 0.10 p.p.m.). Those at 1.0 p.p.m., however, accumulated significantly more cadmium. This author reported uptake of cadmium by crayfish exposed to levels as low as 0.25 and 0.50 p.p.m. but no analyses were conducted to see if rates of uptake were statistically different at these levels (see experiment 4.5). Dethlefsen (1977) also considered cadmium accumulation from low environmental levels (0.005 - 0.10 p.p.m.) and reported a rapid initial uptake of the metal which reached a plateau after 48 hours, and was the result of adsorption onto the external surface of the cuticle. This was followed by a linear uptake of cadmium with time, accumulation now occurring by other means.

The sites of uptake of cadmium by *A. pallipes* were shown to be the gills > cuticle > hepatopancreas > alimentary tract > gonads > muscle (this study). In the majority of the marine Crustacea reported in Table 4.9 the hepatopancreas is the chief site of cadmium accumulation, although exceptions do exist. Zinc accumulation has previously been studied in *A. pallipes* (Bryan, 1967), and for this metal it was found that at low levels of exposure (0.004 p.p.m.) zinc occurred most commonly in the hepatopancreas and gut fluid, whilst very little occurred in the gills. This equates to the normal situation. Upon exposure to 20 p.p.m. zinc, the gills became the chief site of uptake, followed by the hepatopancreas > gut fluid > cuticle > muscle > blood, and it was suggested that a large part of the zinc that was apparently in the gills and cuticle could be explained by adsorption onto their surfaces. From these results it would thus appear that the greater accumulation of cadmium by the gills of *A. pallipes* may be dependent on the external-concentration, rather than on a difference between freshwater and marine crustaceans as might appear to be the case initially.

Most of the marine species had been exposed to low concentrations (but they are more susceptible to cadmium than freshwater species see 3.(iv)). Nevertheless one exposed to a very low cadmium concentration (*Homarus armericanus* - 0.006 p.p.m.) accumulated more metal in the gills than hepatopancreas (Thurberg *et. al.*,1977), and this was also the case for one exposed to a relatively high concentration (*Uca pugilator* - 1.0 p.p.m., Vernberg *et. al.*1974). Conversely, *Penaeus duorarum* exposed to high (5.0 p.p.m.) or low (0.075 p.p.m.) cadmium concentrations consistently was found

to have more cadmium in the hepatopancreas (Nimmo *et. al.* 1977). In this study accumulation of cadmium by *A. pallipes* was consistently seen to be greater in the gills at both high (50.00 p.p.m.) and low (0.25 p.p.m.) external cadmium concentrations, and levels in the gills were of an order of magnitude greater than those in the hepatopancreas. Thus it would appear that no definitive conclusion may be made concerning the sites of uptake of cadmium between either freshwater and marine organisms, or between those exposed to high and low external cadmium concentrations.

The depuration of cadmium by *A. pallipes* was also studied, and it appeared that some removal of cadmium from the gills to other tissues may occur, although the result was not significant. Significant increases of cadmium, however, did occur in the hepatopancreas, gut, and cuticle, as the time in clean water increased. Detectable amounts of cadmium occurred in the muscle by day 21. Total body levels appeared to follow a decreasing trend, but the level after 28 days was still very similar to that of day 0 and it is doubtful whether a significant reduction occurred.

Authors who have studied cadmium depuration report a variety of effects. Dethlefsen (1977) reported that shrimps *(Crangon crangon)* previously exposed to 0.005 and 0.01 p.p.m. cadmium and placed in clean water showed no loss of accumulated metal, whilst those exposed initially to 0.02 p.p.m. showed a loss for the first 3 days but not thereafter. Ray *et. al.* (1980b) reported a decrease in cadmium levels in most tissues after previously exposed shrimp (*Pandalus montagui*) had been transferred to clean water for 57 days. An increase of the level in the hepatopancreas was, however, apparent, and they suggest that this may indicate

the induction of a cadmium binding metalothionein in the hepatopancreas. For the shrimps *Penaeus duorarum* and *Palaemonetes vulgaris*, Nimmo *et. al.*, (1977) reported that cadmium exposed animals transferred to fresh water showed a decrease of the blood and exoskeleton cadmium concentrations, an increase of those in the muscle, whilst the levels in the hepatopancreas were unchanged. Overall 50% of the cadmium was lost in 7 days. Wright and Brewer (1979), working with the crab, *Carcinus maenas*, showed that after exposure to labelled cadmium, and removal to clean water, cadmium would continue to increase in the hepatopancreas, possibly from the haemolymph, although data were not sufficient to give a quantitative estimate. From the haemolymph, cadmium was also lost through the gills, and in urine and faeces, although these losses were masked by larger losses from the gills and cuticle themselves. In clean water about half of the cadmium on the cuticle was lost, probably due to desorption of the metal. In studying the metabolism of zinc in *Carcinus maenas*, *Homarus vulgaris* and *A. pallipes*, Bryan (1966), reported that the metal could be removed in the faeces but that it could not be lost unless the animal was given solid food. During the studies conducted by this author the animals were not fed; the results may have been different if they had been. The initial loss of cadmium from the exoskeleton was probably the result of desorption, but its subsequent increase in the cuticle must have arisen from another process.

The precise method of heavy metal uptake, and its route of translocation through the tissues, and possible subsequent depuration, has not been entirely established. As previously suggested, adsorption onto the surfaces of the gills and cuticle

may explain some of the metal in these tissues. However, in neither case can this be the only explanation. Cadmium was seen to accumulate in the gills well beyond the levels in the water, and no evidence that a plateau was being reached was found (experiments 3 - 5). The depuration experiment (2) showed that cadmium adsorbed onto the surface of the cuticle could be desorbed in 7 days, but thereafter accumulation continued to occur. Thus it is suggested that, as was found by Dethlefson (1977), adsorption is only important in the short term, and in the long term other processes operate. Thus the differences observed between the levels of cadmium in the gills and hepatopancreas of *A. pallipes* cannot be explained simply by adsorption at the gills.

Bryan (1967) suggested that the differences observed for the uptake of zinc by *A. pallipes* from concentrations of 0.004 p.p.m. and 20 p.p.m. (reported above) may be due to the fact that in the latter case, external concentrations exceed blood concentrations. Hence an influx of zinc would occur resulting in the different tissue concentrations and distributions observed from those crayfish exposed to 0.004 p.p.m. zinc, which equates to the normal situation. Wright and Brewer (1979) in their study of the uptake of radiolabelled cadmium by the crab, *Carcinus maenas*, also considered blood (haemolymph) cadmium concentrations. Cadmium was seen to enter the blood very rapidly from solution (within 1 hr), and it was then bound to a haemolymph protein. This effective removal of the cadmium caused a sufficient gradient for the uptake of more cadmium by the blood. Stabilization occurred at a level below that of the environment. Some evidence existed to suggest that the cadmium may then be passed from the haemolymph

into the hepatopancreas. Since only low levels of cadmium were found in the hepatopancreas of *A. pallipes* it is possible that they may have been derived from the blood.

The gills, however, accumulated the most cadmium. They are supplied with large amounts of blood to facilitate respiratory gas exchange (see 5(iv)), and so it is possible that cadmium could pass into the blood from the gills and become bound to haemolymph proteins. The remainder of the exocuticle of the freshwater crayfish is impervious to water and ion movement, and so the gills (and gut) should be the only route of uptake into the blood, which then acts as the medium for redistribution of cadmium within the crayfish.

Crayfish gill tissues are involved in osmoregulatory and respiratory functions and their structure in relation to these functions will be described (see 5(iv)). They therefore 'filter' large volumes of water, and if cadmium is in the external environment this will also pass either through, or into the gills. In 3(iv) it was suggested that cadmium uptake into the gills may occur by the route through which calcium is normally taken up. Thus accumulation of cadmium would occur by an active process albeit 'accidentally', as has been shown to be the case for *Gammarus pulex* (Wright, 1980).

The cellular mechanisms of heavy metal uptake have been reviewed by Fowler *et. al.*, (1981). Lysosomes and membrane bound bodies containing a variety of metals (Hg, Pb, Fe, Cu) have been demonstrated in aquatic invertebrates including crustaceans, but none have so far been demonstrated for cadmium. These bodies are active in the role of metal uptake (by pinocytosis), and

in subsequent storage may serve to detoxify the metals due to their inert form within the lysosome. The accumulation of calcium phosphate granules and concretions have also been implicated in the storage of heavy metals by crustaceans, and particularly the metal cadmium. this would support the view that the calcium uptake mechanism may also serve to accumulate cadmium. The hepatopancreas of crabs is thought to be the site of calcium concretions, which are stored for the transfer of calcium to the exoskeleton at moulting (Becker *et. al.*, quoted in, Fowler *et. al.*, 1981). Thus if cadmium were also in these concretions, and a similar mechanism operated in *A. pallipes*, then the observed increase in the cadmium level of the cuticle (experiment 2) could be explained if the cuticle was still being calcified. Fowler *et. al.*, (1981) have reported up to 810 p.p.m. cadmium in calcium concretions from the hepatopancreas of crabs, and it might also be interesting to examine the cadmium content of gastroliths of crayfish which had been exposed to low levels of cadmium for a long period of time. Within the concretions the cadmium may be in an inert and non-toxic form. Finally, Fowler *et. al.*,(1981) review the evidence for metal binding proteins, and the existence of cadmium binding proteins in crustaceans has been discussed in 3(iv).

Thus a variety of mechanisms may operate within an organism, but to different extents in different tissues. A possible route of cadmium accumulation by the tissues of *A. pallipes* could be:

(i) Uptake at the gills by the mechanisms normally involved in calcium accumulation, followed by activation of the production of metal binding proteins.

(ii) Passage across a diffusion gradient into the blood where

the cadmium becomes bound to haemolymph proteins.

(iii) Transfer to the hepatopancreas and incorporation into calcium concretions, resulting in detoxification of the metal.

(iv) Calcification of the cuticle resulting in redistribution of cadmium from the calcium concretions to the exoskeleton.

(v) Low levels of cadmium also accumulate in the gonads and muscle, the source being the blood.

As to the routes by which cadmium is lost from the body, there is:

(i) Transference to the alimentary tract and elimination with the faeces.

(ii) Although the levels of cadmium in the gills remain high between moults because metal binding proteins prevent further redistribution, the metal may be eliminated when moulting occurs and the old exocuticle is sloughed off. This route of loss is known to occur for lead in crayfish gill tissue (Anderson, 1978; see also 5(iv)).

This hypothesis is consistent with the reported experimental results and with similar studies in the literature. It opens the doors for further detailed studies:

- Do metal binding proteins exist in the gills of *A. pallipes?*
- What levels of cadmium occur in calcium concretions in the hepatopancreas and in the gastroliths?
- Is cadmium elimination enhanced by feeding, such that it may be removed in the faeces?
- Can active cadmium uptake be prevented by suitable inhibitors, e.g., Dinitrophenol, which is known to inhibit calcium uptake?
- Is cadmium in the gills eliminated at moulting?

Finally, it is suggested that the T.E.M. studies would be worth repeating. This author was unable to demonstrate any gross changes in tissue structure, but the above discussion indicates that many fine changes may occur. Examination for the presence of lysosomes should be conducted, particularly in the gills, where pinocytosis may be a mechanism of heavy metal uptake. The hepatopancreas should be examined for calcium concretions, and these if present should be extracted (see Fowler *et.al.*1981), and examined for cadmium. X-ray microanalysis of T.E.M. thin sections of gill tissue, and hepatopancreas tubules, was conducted in areas where it was felt that cadmium may be found (this study). No evidence of cadmium was found despite the fact that this study has shown that high concentrations of the metal certainly occur in the gills. It is felt that the lack of expertise of the author in the field of electron microscopy, and the lack of time available to become acquainted with all the techniques may explain the negative results. Thus it is suggested that this may form the basis of a complete programme of study for someone who is able to direct all of their energies in this complex field.

TABLE 4.1 UPTAKE OF CADMIUM BY MALE AND FEMALE CRAYFISH (10 p.p.m., 7 DAYS EXPOSURE)

TISSUE TYPE	TISSUE CADMIUM CONTENT, mg $Cd^{2+}g^{-1}$						COMPARISON OF THE SEXES		
	MALES			FEMALES					
	RANGE	MEAN	S.E.	RANGE	MEAN	S.E.	t	df	P
Cuticle	0.09 - 0.15	0.12	0.02	0.08 - 0.29	0.20	0.06	1.27	4	>0.1
Gills	0.68 - 1.22	1.03	0.18	0.68 - 2.65	1.41	0.62	0.58	4	>0.1
Hepatopancreas	0.03 - 0.27	0.12	0.08	0.01 - 0.09	0.05	0.02	0.83	4	>0.1
Alimentary tract	0.01 - 0.06	0.03	0.01	0.01 - 0.07	0.04	0.03	0.15	4	>0.1
Gonads	0.00 - 0.09	0.03	0.03	0.00 - 0.00	0.00	0.00	1.00	4	>0.1
Tail muscle	0.00 - 0.01	0.006	0.003	0.00 - 0.01	0.006	0.003	0.00	4	>0.1

TABLE 4.2 DEPURATION OF CADMIUM FROM THE TISSUES OF MALE CRAYFISH

(10 p.p.m., 7 DAYS EXPOSURE, 28 DAYS IN CLEAN WATER)

TISSUE TYPE	TIME (DAYS)	TISSUE CADMIUM CONTENT mg Cd^{2+} g^{-1} (MALES ONLY)			1 WAY ANOVAR (TIME)		
		RANGE	MEAN	S.E.	F	df	P
CUTICLE	7 EXP.	0.05 - 0.19	0.11	0.04	3.95	4, 10	<0.05
	7 F/W	0.04 - 0.13	0.07	0.03			
	14	0.04 - 0.08	0.07	0.01			
	21	0.03 - 0.17	0.08	0.05			
	28	0.02 - 0.16	0.10	0.04			
GILLS	7 EXP.	0.75 - 1.01	0.95	0.10	1.87	4, 10	>0.05
	7 F/W	0.52 - 1.25	0.81	0.22			
	14	0.15 - 0.47	0.28	0.10			
	21	0.26 - 1.01	0.56	0.23			
	28	0.21 - 1.27	0.57	0.35			
HEPATO-PANCREAS	7 EXP.	0.006 - 0.02	0.01	0.005	8.22	4, 10	<0.01
	7 F/W	0.004 - 0.02	0.01	0.005			
	14	0.02 - 0.03	0.02	0.003			
	21	0.02 - 0.05	0.03	0.009			
	28	0.06 - 0.17	0.10	0.04			
ALIMEN-TARY TRACT	7 EXP.	0.006 - 0.06	0.03	0.02	9.41	4, 10	<0.01
	7 F/W	0.00 - 0.04	0.01	0.01			
	14	0.004 - 0.02	0.01	0.005			
	21	0.005 - 0.03	0.02	0.008			
	28	0.02 - 0.08	0.05	0.02			
GONADS	7 EXP.	0.00 - 0.01	0.003	0.003	-	-	-
	7-28 F/W	0.00 - 0.00	0.00	0.00			
TAIL-MUSCLE	7 EXP.	0.00 - 0.002	<0.001	-	-	-	-
	7 F/W	0.00 - 0.009	<0.001	-			
	14	0.00 - 0.008	<0.001	-			
	21	0.00 - 0.005	0.003	0.002			
	28	0.00 - 0.010	0.002	0.005			

TABLE 4.3a UPTAKE OF CADMIUM BY THE GILLS OF CRAYFISH EXPOSED TO CADMIUM FOR AN INCREASING TIME PERIOD ((i) 1 p.p.m., (ii) 10 p.p.m. EXTERNAL CONCENTRATION)

TIME OF EXPOSURE (DAYS)	TISSUE CADMIUM CONTENT mg Cd^{2+} g^{-1} (TCd)					
	(i) 1 p.p.m.			(ii) 10 p.p.m.		
	RANGE	MEAN	S.E.	RANGE	MEAN	S.E.
1	0.17 - 0.30	0.21	0.02	0.11 - 0.43	0.24	0.06
3	0.03 - 0.32	0.17	0.04	0.63 - 2.76	1.16	0.32
6	0.12 - 0.71	0.44	0.09	1.67 - 2.95	2.20	0.24
9	0.47 - 1.18	0.76	0.10	2.88 - 4.15	3.58	0.21

TABLE 4.3b RESULTS OF REGRESSION ANALYSES ON THE RAW DATA FOR TABLES 4.3a, 4.4 (CORRECTED TO 2 DECIMAL FIGURES)

CONCENTRATION	EQUATION OF LINE	r	F	P
1 p.p.m.	TCd = 0.07. TIME + 0.05	0.79	34.67	<0.01
10 p.p.m.	TCd = 0.41. TIME - 0.15	0.91	102.56	<0.01
1 p.p.m. - 50 p.p.m.	TCd = 0.13. CONC. - 0.20	0.98	633.38	<0.01

TABLE 4.4 UPTAKE OF CADMIUM BY THE GILLS OF CRAYFISH EXPOSED TO CADMIUM OF INCREASING CONCENTRATIONS FOR 3 DAYS

EXTERNAL CONCENTRATION	TISSUE CADMIUM CONTENT mg Cd^{2+} g^{-1} (TCd)		
	RANGE	MEAN	S.E.
1 p.p.m.	0.03 - 0.32	0.17	0.04
5 p.p.m.	0.25 - 0.57	0.39	0.06
10 p.p.m.	0.63 - 2.76	1.16	0.32
25 p.p.m.*	1.55 AND 1.72	1.64	0.09
50 p.p.m.	5.93 - 7.65	6.38	0.17

* Based on only two sets of gills, excluded from regression.

TABLE 4.5 UPTAKE OF CADMIUM BY NANPANTAN MALE CRAYFISH OVER 28 DAYS

TISSUE TYPE	TIME (DAYS)	TISSUE CADMIUM CONTENT mg Cd^{2+} g^{-1}, WITH EXTERNAL CONCENTRATIONS OF (p.p.m.):						
		0.25	0.5	2.5	5.0	10.0	20.0	30.0
CUTICLE	7	0.02	0.03	0.04	0.10	0.10	0.33	0.18
	14	0.006	0.01	0.03	0.07	0.17	1.09	0.27
	21	0.02	0.02	0.04	0.12	0.58	0.42	0.39
	28	0.02	0.08	0.11	0.18	1.19	0.52	0.71
GILLS	7	0.29	0.41	1.35	1.59	3.20	11.73	6.01
	14	0.27	0.52	1.38	3.33	5.34	11.57	10.03
	21	0.37	0.61	1.75	3.30	10.67	7.91	10.86
	28	0.73	1.82	2.64	5.86	17.34	14.41	16.45
HEPATO-PANCREAS	7					0.15		
	14						0.15	0.19
	21			0.007	0.02		0.04	0.09
	28	0.04		0.02	0.24	0.20	0.18	0.23
ALIMENTARY TRACT	7					0.007		
	14						0.22	0.05
	21				0.03	0.21	0.03	0.05
	28	0.02		0.14	0.27	0.07	0.10	0.05
GONADS	7					0.02		
	14		0.05			0.02		0.24
	21					0.78		
	28	0.01	0.18		0.07	0.26	0.26	0.11
TAIL-MUSCLE	7			0.001			0.007	0.06
	14						0.09	0.02
	21				0.005	0.02	0.03	0.05
	28	0.008	0.11	0.02	0.06	0.05	0.04	0.10

TABLE 4.6 REMOVAL OF CADMIUM FROM SOLUTION OVER 7 DAYS (p.p.m.)

NOMINAL CONC: 0.0*	0.25	0.5	2.5	5.0	10.0	10.0*	20.0	30.0
CALCULATED CONC: 0.0	0.28	0.51	2.45	4.96	10.09	9.94	19.86	29.69

TIME(DAYS)	MEASURED CONC. (CUMULATIVE % CHANGE)								
0	0.0	0.21	0.50	2.34	4.66	9.98	9.12	19.20	27.5
1	0.0	0.22(+4.8)	0.48(-4.0)	2.31(-1.3)	4.70(+0.9)	9.00(-9.8)	8.48(-7.0)	14.20(-26.0)	24.0(-12.7)
2	0.0	0.21(0.0)	0.48(-4.0)	2.33(-0.4)	4.48(-3.9)	8.68(-13.0)	8.48(-7.0)	15.10(-21.4)	22.0(-20.0)
3	0.0	0.19(-9.5)	0.47(-6.0)	2.13(-9.0)	4.11(-11.8)	9.00(-13.0)	8.48(-7.0)	15.00(-21.9)	21.0(-23.6)
4	0.0	0.17(-19.0)	0.45(-10.0)	2.01(-14.1)	4.01(-14.0)	8.00(-19.8)	8.04(-11.8)	14.10(-26.6)	21.1(-23.0)
7	0.0	0.15(-28.6)	0.44(-12.0)	2.00(-14.5)	3.86(-17.2)	8.40(-15.8)	7.80(-14.5)	14.40(-25.0)	20.9(-24.0)

* Controls, no animals.

TABLE 4.7 CONTROLS. TISSUE METAL ION CONCENTRATIONS FOR ANIMALS FROM EACH OF THE NANPANTAN, LEEN, AND MARKFIELD POPULATIONS, AND ALSO ENVIRONMENTAL LEVELS OF METALS

POPULATION	TISSUE/WATER	TISSUE METAL CONCENTRATION, mg METAL g^{-1} ±1 S.E.						
		Cd	Zn	Mn	Cu	Fe	Ni	Pb
NANPANTAN	All tissues	0.01	-	-	-	-	-	-
	Tap water	0.01	-	-	-	-	-	-
RIVER LEEN	Cuticle	<0.01	0.06 ± 0.02	0.11 ± 0.04	0.05 ± 0.02	0.19 ± 0.09	0.03 ±0.01	0.09 ±0.06
	Gills	<0.01	0.33 ± 0.17	0.10 ± 0.02	0.46 ± 0.08	1.20 ± 0.61	0.07 ±0.02	0.46 ±0.19
	Hepatopancreas	<0.01	0.19 ± 0.09	0.08 ± 0.03	0.14 ± 0.03	0.25 ± 0.10	0.01 ±0.01	0.08 ±0.03
	Al. tract	<0.01	0.23 ± 0.06	0.56 ± 0.31	0.08 ± 0.03	1.31 ± 0.64	0.05 ±0.02	0.21 ±0.13
	Gonads	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Tail muscle	<0.01	0.16 ± 0.02	<0.01	0.06 ± 0.01	0.10 ± 0.04	<0.01	0.05 ± 0.01
Study area	water	<0.01	0.01	0.06	0.06	0.30	<0.01	<0.01
confluence	water	0.28	0.76	0.54	0.37	2.20	0.05	0.20
MARKFIELD QUARRY	Cuticle	<0.01	0.09 ± 0.03	0.18 ± 0.08	0.06 ± 0.01	0.21 ± 0.05	0.04 ±0.01	0.10 ± 0.06
	Gills	<0.01	0.52 ± 0.20	0.17 ± 0.08	0.39 ± 0.09	1.80 ± 0.51	0.17 ±0.04	0.66 ± 0.54
	Hepatopancreas	<0.01	0.20 ± 0.10	0.11 ± 0.06	0.09 ± 0.03	0.26 ± 0.11	0.01	0.09 ± 0.02
	Al. tract	<0.01	0.21 ± 0.05	0.41 ± 0.22	0.09 ± 0.04	1.17 ± 0.51	0.01 ±0.01	0.25 ± 0.12
	Gonads	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Tail muscle	<0.01	0.18 ±0.04	<0.01	0.16 ±0.08	0.16 ±0.08	<0.01	0.07 ±0.02
study area	water	<0.01	0.01	0.04	0.20	0.20	<0.01	<0.01

TABLE 4.8 THE LEVELS OF VARIOUS METALS FOUND TO OCCUR NATURALLY IN SOME FRESHWATER CRAYFISH

ORGANISM	LOCATION	TISSUES EXAMINED	METAL CONTENT, $\mu g.g^{-1}$ DRY WEIGHT				REFERENCE
			Cd	Cu	Pb	Zn	
Austropotamobius pallipes	River Leen	Gills	<1.0	460.0	460.0	190.0	Author
		Hepatopancreas	<1.0	140.0	80.0	230.0	
A. pallipes	Markfield Quarry	Gills	<1.0	390.0	660.0	520.0	Author
		Hepatopancreas	<1.0	90.0	90.0	200.0	
A. pallipes	Midlands	Gills	6.0	43.0	111.0	128.0	Tolba, unpublished
		Hepatopancreas	5.0	459.0	15.0	92.0	
A. pallipes	Surrey	Gills	-	26.0	-	8.0	Bryan, 1967
		Hepatopancreas	-	335.0	-	109.0	
Orconectes spp.	Fox R. Illinois	All body	1.6	86.6	25.7	107.1	Anderson, 1977
Procambarus spp.	Fox R.	All body	2.8	58.1	15.7	64.7	Anderson, 1977
Cambarus spp.	Fox R.	All body	1.7	94.8	15.6	93.4	Anderson, 1977

TABLE 4.9 THE UPTAKE OF CADMIUM BY CRUSTACEA (ZINC ALSO FOR *A. PALLIPES*)

ORGANISM	Cd^{2+} CONC. (p.p.m.)	TIME OF EXPOSURE (DAYS)	TISSUES AFFECTED	DEPURATION STUDIED?	NOTES	REFERENCE
FRESHWATER:						
A. pallipes	0.25-50.0	1-28	G>C>Hp>A.T.>G_N>M	YES	See text.	Author
A. pallipes	0.004(^{65}Zn)	32	Hp>A.T.(fluid)>G_N>M>G>C	YES	Represents normal situation.	Bryan, 1967
	20.0(^{65}Zn)	32	G>Hp>A.T.(fluid)>C>M>B	YES	Removal occurs in faeces.	
Orconectes propinquus	0.01-1.0	1.5-109.5hrs.	Total body	-	^{109}Cd used. Max internal Cd^{2+} = 534 p.p.m.	Gillespie *et.al.*1977
Gammarus pseudolimnaeus	1.0	28	Total body	-	Cd^{2+} internal = 103 x Cd^{2+} Ext.	Spehar *et. al.*, 1978
MARINE:						
Homarus americanus	0-0.006	30	G>>Hp, M = N/D	-	Accumulation = time/conc. dependent.	Thurberg *et.al.*,1977
H. americanus	-	-	90% Hp>>G, Gg	-	Polluted natural waters.	Ray *et.al.*, 1981a
Penaeus duorarum	0-5.0	4	Hp>C>M>B	YES	Accumulation = time/conc. dependent.	Nimmo *et.al.*, 1977
Palaemonetes vulgaris	0-5.0	4	Hp>C>M>B	YES	Accumulation = time/conc. dependent.	Nimmo *et.al.*, 1977
Crangon crangon	0.005-0.10	20	Hp>G>E.S.>M>G_N>C	YES	Rapid initial uptake,thereafter accumulation= time dependent.	Dethlefsen, 1977
Penaeus kerathurus	0.8	20	Hp>>M	-		Establier *et.al.*1978
Pandalus montagui	0.037	14	Hp>C>Eg>M	YES		Ray *et.al.*, 1980b
Crangon septemspinosa	-	-	Total body	-	Polluted sediments. Accumulation occurs.	Ray *et.al.*, 1981b
Callinectes sapidus	0.0001	8	C>Hp>G	-	Other tissues = no accumulation.	Hutcheson, 1972
	11.14	8	G>Hp>C>G>E.S.>M	-	Carapace Cd reaches saturation.	
Uca pugilator	0.18-1.0	3	G>>Hp	-	Cd^{2+} transferred from G→Hp.	Vernberg *et.al.* 1974

NOTES: G = gills, C = cuticle, Hp = hepatopancreas, A.T. = alimentary tract, G_N = gonads, M = muscle, B = blood, E.S. = eye stalk, Eg = eggs Gg = green gland, N/D = Not detectable.

FIG. 4.1 TO SHOW THE DEPURATION OF CADMIUM BY TISSUES OF *A. PALLIPES* OVER 28 DAYS IN CLEAN WATER, FOLLOWING EXPOSURE TO 10 p.p.m. Cd^{+2} FOR 7 DAYS.

The figure shows the amount of cadmium in each tissue on days 0 (7 days exposure), 7, 14, 21 and 28. Standard error bars have not been included since they would complicate the figure, but are available from Table 4.2. Notice that the amount of cadmium in the gills is of an order of magnitude greater than most of the other tissues, and two orders of magnitude for the gonads and tail muscle - hence the change of scale. The total body burden is simply derived by adding the amounts in each tissue for each occasion.

Fig. 4.1

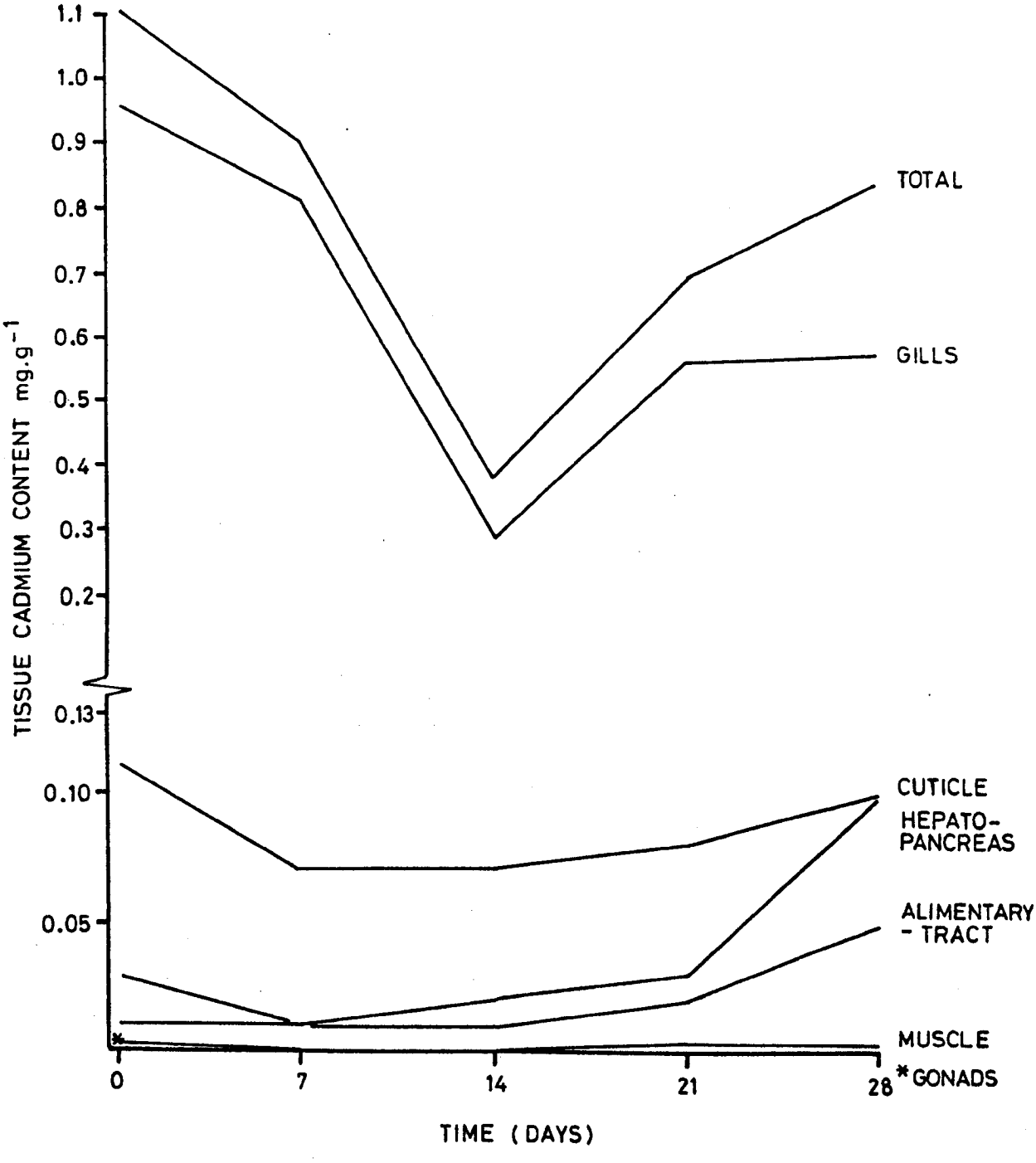

1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.13
0.10
0.05
TISSUE CADMIUM CONTENT mg.g⁻¹
TOTAL
GILLS
CUTICLE
HEPATO-PANCREAS
ALIMENTARY - TRACT
MUSCLE
*GONADS
0
7
14
21
28
TIME (DAYS)

FIG. 4.2 ACCUMULATION OF CADMIUM BY CRAYFISH GILLS WITH INCREASING TIME OF EXPOSURE

The accumulation of cadmium in the gills of crayfish exposed to 1 p.p.m. and 10 p.p.m. cadmium is shown on days 1, 3, 6, and 9, ±1 S.E.. The best fit regression line (Table 4.3b) has been applied to the data points.

FIG. 4.3 ACCUMULATION OF CADMIUM BY CRAYFISH GILLS WITH INCREASING EXTERNAL CADMIUM ION CONCENTRATIONS

The accumulation of cadmium after exposure to a range of cadmium ion concentrations for 3 days is shown ±1 S.E.. The best fit regression line has been applied to the data points (Table 4.3b), but does not include the result of 25 p.p.m. which consisted of only two sets of data.

Fig. 4.2

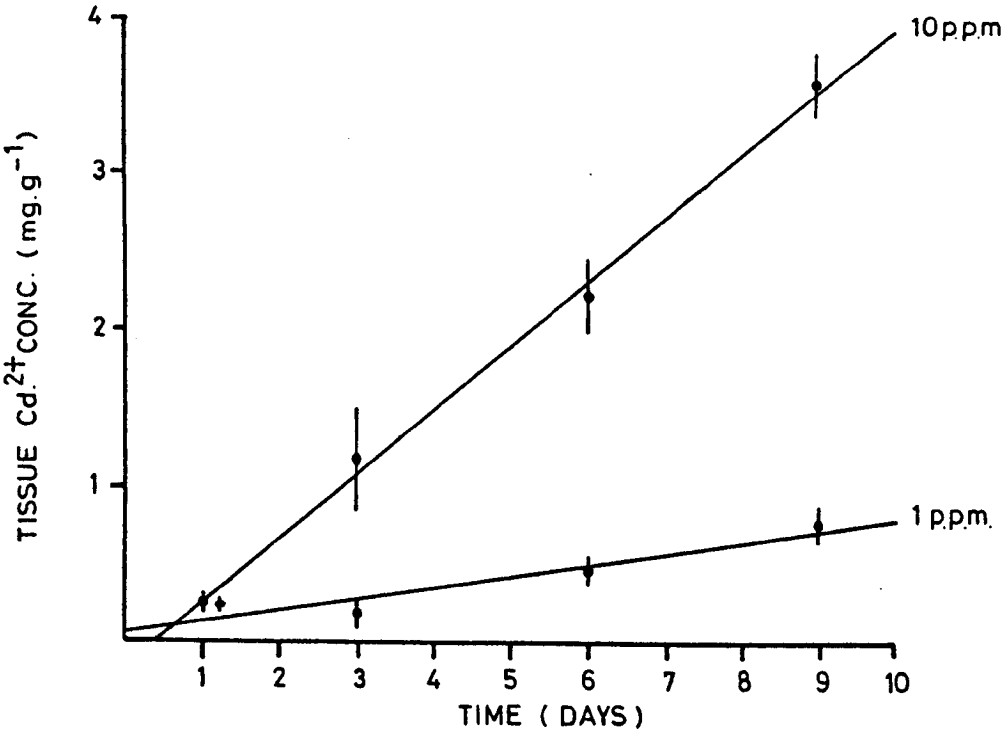

10 p.p.m.
1 p.p.m.
TISSUE Cd.2+CONC. (mg.g-1)
TIME (DAYS)
1
2
3
4
5
6
7
8
9
10

Fig. 4.3

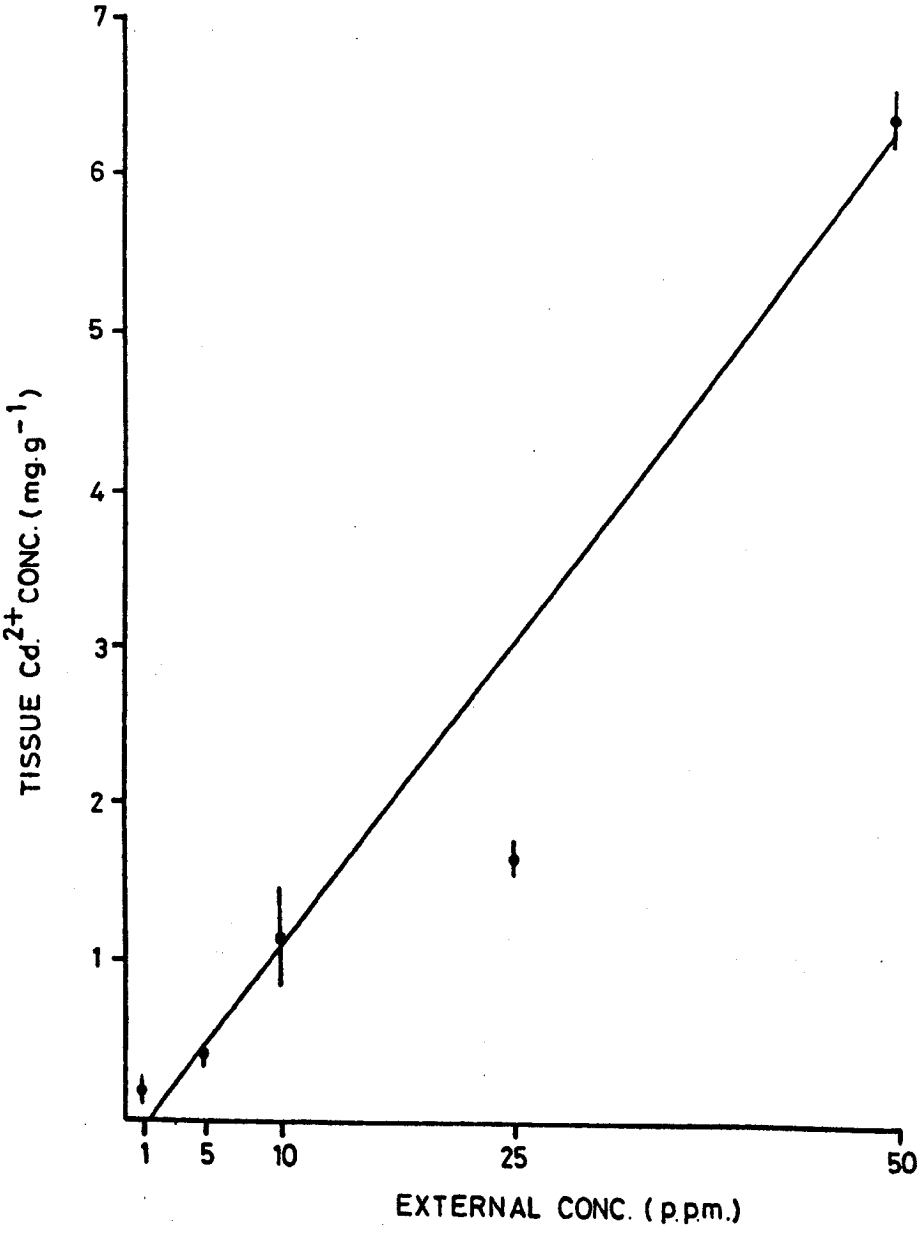
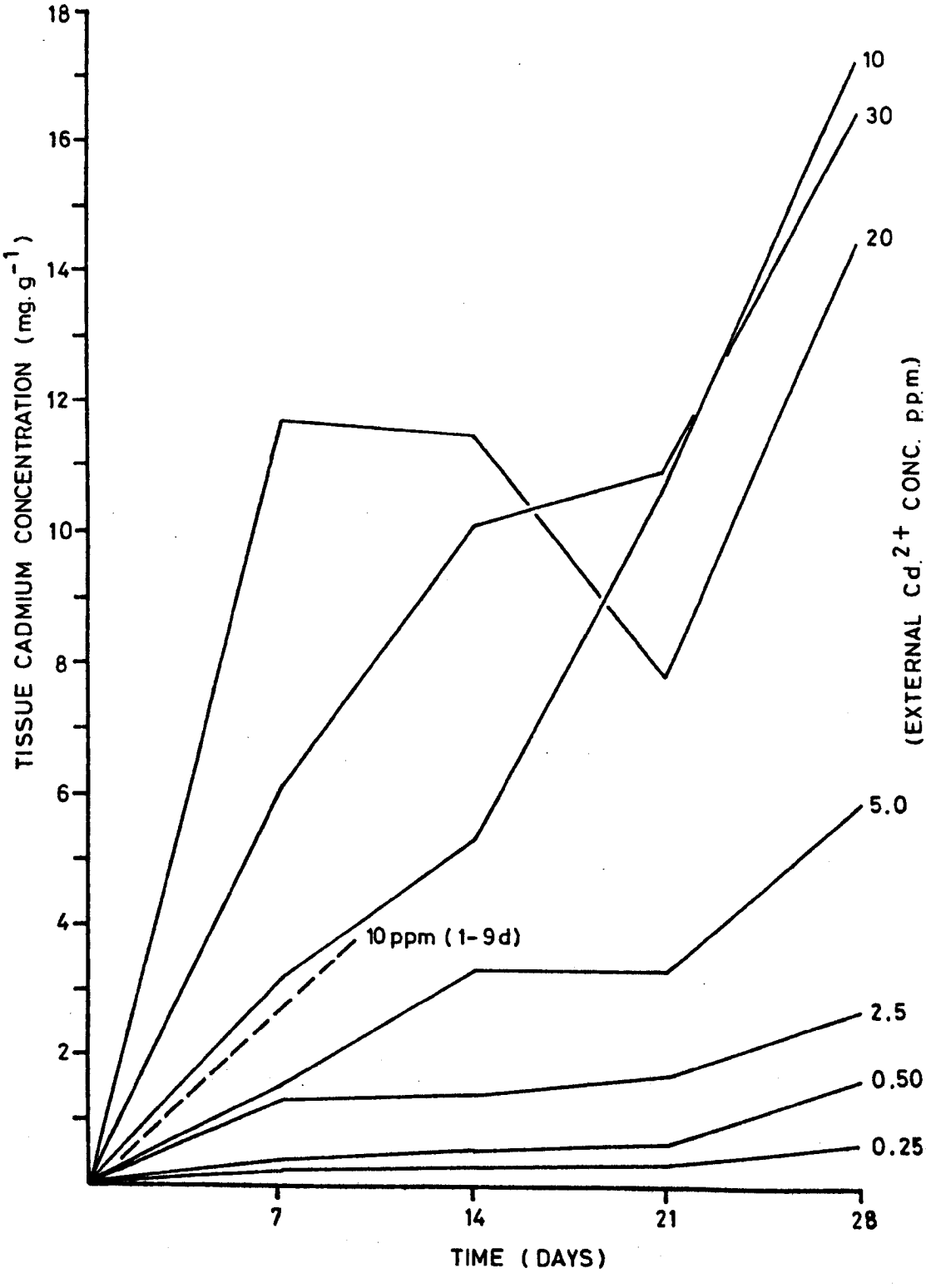

TISSUE Cd.2+CONC. (mg.g-1)
EXTERNAL CONC. (p.p.m.)
1
5
10
25
50

FIG. 4.4 GILL TISSUE Cd^{2+} CONCENTRATIONS AFTER EXPOSURE OF CRAYFISH TO A RANGE OF EXTERNAL Cd^{2+} CONCENTRATIONS FOR 28 DAYS



4.2 LINDANE ACCUMULATION IN THE TISSUES OF *A. PALLIPES*

4.2(i) MATERIALS AND METHODS

In order to establish the dynamics of Lindane accumulation by *A. pallipes* a range of experiments similar to those described in 4.1 should be conducted. However, since sublethal levels are represented by only very low concentrations of toxicant (which would be at the limits of detection for Gas-Liquid-chromatographic techniques), radiolabelled material must be employed. Owing to the cost of such material, only a limited number of experiments could be conducted by this author, and those chosen were:

1. An initial experiment to establish the sites of uptake, and examine for sexual differences.
2. Accumulation of Lindane over a 12 day period.

Markfield Quarry stock were used for each experiment and they were initially subject to the usual pre-experimental procedure (see 2). Only winter intermoult animals were used, and all the females employed were non-ovigerous. Owing to the cost of radio-labelled material only very small quantities could be used, and so experiments were conducted in only 150 mls of water in individual 800 mls plastic beakers (previously soaked to remove PCBs, see 3) containing only 150 mls of water and toxicant. It was thus necessary to aerate the water despite the danger of volatilization of pesticide, since it was not possible to change the water and toxicant daily, as was the procedure with unlabelled material. Aeration was achieved through cut-off plastic pipettes held in place by a plastic petri dish inserted into the top of the beaker. The petri dish also served to present splashing of labelled water, and it stopped crayfish escaping. Plastic was

used in preference to glass since disposal of contaminated material was easier. The experiments were conducted in the cold room at 10°C ±1°C.

1. Uptake of Lindane by male and female crayfish.

3 male (C.L. 34.3 ±1.5 mm) and 3 female (C.L. 32.6 ±3.7mm) crayfish were each exposed to 15% of the labelled material available, which was equivalent to 0.03 p.p.m. Lindane when in 150 mls of water (calculated in the manner described below). They were exposed to toxicant for 7 days, during which time the pH of the water varied between 7.10 and 7.85. After this period they were flushed in clean water to remove toxicant from the surface of the cuticle, and the cuticle, gills, hepatopancreas, alimentary tract, gonads, and tail muscle were dissected out into preweighed glass 'counting' vials. The wet weight was taken, and the tissues were then solubilized to enable the amount of radioactivity to be counted. A comparison of the results of male and female crayfish for each tissue was conducted using the t-test.

A 0.5 ml sample of water was taken from each beaker each day in order to monitor any changes in the ambient level of Lindane, and a beaker containing 150 mls of water plus 10% of the labelled Lindane but no crayfish, was also monitored.

2. Uptake of Lindane by crayfish over an increasing time period.

7 male and 8 female crayfish were used for this experiment (C.L. 27.5 ±2.4 mm). They were each exposed to 6.25% of the total labelled Lindane available, which was equivalent to 0.014 p.p.m. Lindane. This was made up to 0.034 p.p.m. by adding cold Lindane. 3 animals were removed, killed, and dissected into counting vials for tissue solubilization on each of days 1, 3, 6, 9 and 12.

All of the tissues previously monitored were studied. The pH of the water in the beakers varied between 7.10 and 7.55 throughout the 12 day period. One way analysis of variance was employed to find if there was any significant effect occurring with time, and within any of the tissues. A Texas TI-59 programmable calculator was used, and precise P values were calculated. A 0.5 ml sample of water was taken from each beaker on each sampling occasion, and also from a further beaker which contained 150 mls of water plus the last 6.25% of the labelled material. No animal was in this beaker.

EXPERIMENTAL PROCEDURE

Two vials containing 10 μCi of λ[u-^{14}C] Benzenehexachloride (Lindane) each, were obtained from Amersham International Ltd., one for each experiment. The labelled material was a beta-emitter. The Lindane was supplied dissolved in hexane, but since this solvent is imiscible with water it was driven off under vacuum over a water bath heated to 50°C. The labelled Lindane was then redissolved in acetone. 1 ml of acetone was used in the first experiment, and 2 ml in the second. The method of calculating how much material to add to each beaker was as follows:

Suitable levels of detection are 5,000 - 10,000 counts per minute (c.p.m.) when using liquid scintilation counting (LSC) techniques.

Now, 1 Ci = 3.7×10^{10} disintegrations per second (D.P.S.)

and, 1 Ci = $3.7 \times 10^{10} \times 60 \times 0.4$ c.p.m.

(assuming the efficiency of the counter to be 40%)

so 10μCi = 8.88×10^{6} c.p.m. (per volume of acetone).

If 8,800 c.p.m. is taken as the desired level, then the volume

of acetone containing 10 μCi Lindane may safely be diluted by 1,000. Thus 10 μCi is sufficient to make 1 litre of stock solution. In experiment 1, 6 x 150 mls (900 mls) contained 0.15 ml of the labelled material, whilst the final 0.1 ml was used as a blank, as described. In actual fact the counting efficiency of the machine proved to be greater than 40% and the initial counts were high, suggesting that about 3 times as many animals could have been exposed to toxicant. Thus in experiment 2 it was possible to use 15 animals and smaller quantities of labelled material.

A Hewlett-Packard Scintilation counter was used to count all of the samples. It records photon emission brought about by the activity of the radiolabelled material upon the scintilation fluid used to carry it. The scintilant chosen was Aquasol (New England Nuclear). It is a xylene based universal L.S.C. cocktail, chosen since it was suitable for both the water samples and the solubilized tissues. Hughs and Hughs S.V.1 Mark II scintilation vial inserts were used for the water samples since they enabled the minimum quantities of water to be analysed. 3 mls of scintilant were used to 0.5 mls of water. Tissue solubilization was conducted in the counting vials themselves, and NCS (Trade Mark) tissue solubilizer, available from Amersham International, was used (see Rapkin, 1969). The tissues must be dissolved wet (hence wet weights), and those such as the cuticle which contain relatively little water must have water added. 6 parts of NCS were used to 1 part of tissue (or 1 ml NCS, whichever was the greater), and the samples were then heated to 50°C overnight in an oven to aid solubilization. After cooling, the scintilant was added to the counting vials and the samples were counted.

Tissue which has been solubilized often causes quenching, i.e. the total effect of those phenomena which result in reduced photon output from the sample (see Rapkin, 1970). Hence it is necessary to produce a 'quench-curve'. The Hewlett-Packard counter has programmes which may be set to adjust each sample and allow for factors such as the 'background-noise'. Hence a blank containing only scintilation fluid is counted first and the results of this are subtracted from the results of each sample. Set to automatic the machine then counts each sample, and also an internal standard, and produces a value of the counting efficiency (known as the A.E.S. ratio) of that sample. The efficiency for each sample should be identical, but due to quenching caused by colouring, it generally is not. Thus all of the samples are put through the machine and a value of both c.p.m. and the A.E.S. ratio is obtained. A selection of samples covering the full range of counting efficiencies are then chosen (0.06 to 0.83 were chosen in the case of this study). A standard is prepared, ideally using the original emitter, but since all the labelled Lindane had been used another ^{14}C emitter of similar strength was chosen (L-Valine). A certain quantity of the standard is then added to each of the chosen range of samples and also to scintilant alone. The samples are then put through the machine again and recounted. A value for the A.E.S. ratio is again obtained, and the percentage recovery may be estimated for each sample by comparison with the standard in scintilant alone. The percentage recovery is then plotted against the A.E.S. ratio, and the best line fitted to the points (Fig. 4.5). It is then possible to correct each individual result obtained by applying the appropriate recovery factor obtained

from the 'quench-curve', since a value for the A.E.S. for each sample is known.

The results after correction may be divided by the wet weight of tissue giving c.p.m. mg^{-1} wet weight. This was sufficient to enable the results of experiment 1 to be compared, and analysis was not taken further. In experiment 2 the results were calculated as μg of total Lindane (Hot and cold) per g^{-1} wet weight of tissue, since this is a more meaningful expression than c.p.m. mg^{-1}. Also 1 $\mu g g^{-1}$ is equivalent to 1 p.p.m., i.e. 1 part of Lindane per million parts of tissue. The method of calculation is given below. In addition it was felt that it would be valuable to see what proportion of the total Lindane in all the tissues was in each individual tissue. This proportion was calculated. Standard errors were not calculated since only the mean results were analysed, but they would be of the same proportion as in the previous description of the results. Finally, it was also decided to express the results as a concentration factor, i.e. c.p.m. mg^{-1} of tissue ÷ c.p.m. μl^{-1} of water. In this way accumulation by the tissues was related to the level of Lindane in the ambient solution, particularly valuable in this study since a decline in the ambient levels was seen to occur. A corresponding decline of Lindane, in p.p.m., in the tissues occurred probably due to excretion. This may not have occurred had it been possible to maintain constant levels of toxicant. Thus by expressing the results as a concentration factor, the parameter studied is independent of changing external concentrations. Other authors have also expressed their results as a concentration factor (e.g. see 4.2(iii)).

EXAMPLE CALCULATIONS

(i) The amount of labelled material added per beaker.

λ [u-^{14}C] BHC supplied contains 279 μCi mg^{-1}

$\therefore$ 1μCi $\equiv \frac{1}{279}$ mg of ^{14}C. Lindane

$$10\mu Ci \equiv 3.584 \times 10^{-2} \text{ mg} \quad -(1)$$

and 1 c.p.m. = 4.505×10^{-7} μCi (calc. as previously) -(2)

In experiment 2. Avge. total c.p.m. per beaker,

$= 1.3021 \times 10^{6}$ c.p.m.

From (1) and (2) this is = 2.1024×10^{-3}mg ^{14}C. Lindane per container.

and $\div$ 150 mls $= 1.40 \times 10^{-5}$ mg. ml^{-1}

$= 1.40 \times 10^{-2}$ mg. l^{-1}

and mgl^{-1} = p.p.m.

so, there is: 0.014 p.p.m. labelled Lindane per container.

In experiment 2 this was made up to 0.034 p.p.m. total Lindane by adding cold Lindane. This was done to ensure that sufficient uptake would occur to be detectable. If excretion of Lindane does occur then it was feared that using only 0.014 p.p.m. the rate of uptake may not greatly exceed the rate of depuration, since this is a 'safe level' of toxicant (see 3(iv)). The calculation for experiment 1 was similar, but no cold Lindane was added.

(ii) Calculation of the total c.p.m. in the water in each beaker.

A 0.5 ml sample was taken on each occasion.

$\therefore$ on Day 1, total c.p.m. per beaker = 150 ml x c.p.m. x 2

on Day 2, total c.p.m. per beaker = 149.5 ml x c.p.m. x 2.

on Day 3, total c.p.m. per beaker = 149 ml x c.p.m. x 2, etc.

(iii) To calculate results as μg Lindane g^{-1} wet weight.

1 c.p.m. = 4.505×10^{-7} μCi as above

1 μCi = 3.584×10^{-3} mg ^{14}C. Lindane as above

$\therefore$ 1 c.p.m. = $4.505 \times 3.584 \times 10^{-10}$ mg ^{14}C. Lindane.

= 16.146×10^{-7} g ^{14}C. Lindane.

so, $\frac{\text{c.p.m.} \times 1.6146 \times 10^{-8}\ \mu g}{\text{wet weight in g.}} = \mu g.\ g^{-1}$

but this is only hot Lindane, and cold Lindane is presumed to have been taken up also in direct proportion to the amounts of hot and cold Lindane used, i.e. 0.014:0.020 p.p.m. Thus the hot Lindane represents 41.21% of the total Lindane, so dividing the result obtained above by 0.4121 gives the total Lindane taken up into the tissues expressed as $\mu g.g^{-1}$, or p.p.m.

Note. The concentration factor is not derived by dividing the result of p.p.m. Lindane in the tissues by 0.034 p.p.m. since that concentration only applies to Day 0. The concentration factor is derived for each animal individually based on the c.p.m. mg^{-1} of its tissues, and upon the c.p.m. μl^{-1} of water from the particular beaker in which that animal was being held.

4.2(ii) RESULTS

The results of experiment 1 are expressed in Table 4.10. After 7 days there was no significant difference in the amounts of labelled Lindane taken up into the tissues of males and females. This was true for all tissues ($P > 0.1$). Radioactivity was detectable in all tissues examined, and the order of accumulation was found to be: hepatopancreas > alimentary tract > gills/gonads > cuticle > muscle. The amount present in the hepatopancreas was of an

order of magnitude greater than the amounts in the other tissues.

Table 4.11 and Fig. 4.6 show the loss of radioactive material from the experimental beakers during experiments 1 and 2. Considerable variability is expressed for each data point representing beakers containing a crayfish. Owing to the cost of labelled material it was only possible to run one control test for each experiment and so no valid statistical comparisons may be made. However, it is apparent that the control beakers without animals lost considerably less ^{14}C. Lindane from solution than those containing animals, and despite the variation shown, control data points were always outside the S.D. of those data points for beakers with animals. This indicates that the control results are significantly different. It is therefore reasonable to conclude that the loss of radioactivity in experimental beakers is partly due to accumulation of Lindane by the tissues of the crayfish. That some loss of radioactivity occurs in the control beakers (indeed, a large loss by Day 7 in experiment 1) indicates that Lindane is also being lost from solution by other means, such as adsorption onto the surface of the beaker, and volatilization. Loss of ^{14}C. Lindane from the experimental beakers is seen to occur rapidly initially, and then a plateau is reached as the rate of loss declines.

The results of experiment 2 are expressed in the 3 ways described previously (4.2(i)). Table 4.12 gives the results as μg Lindane (hot and cold) g^{-1} wet weight of tissue, and the results of the 1 way Anovar are also shown. Figs. 4.7a and b illustrate these results. The results which express the amount of Lindane found in each tissue as a proportion of the total radioactivity

recorded also occur in Table 4.12, and are illustrated in Fig. 4.8. Fig. 4.9 illustrates the results expressed in Table 4.13, which describe the accumulation of ^{14}C. Lindane as a concentration factor.

Lindane is seen to accumulate in all the tissues, but the relative proportion in each tissue varies with time of exposure. This is best illustrated by Fig. 4.8. The hepatopancreas consistently is the major site of accumulation with between 60% and 90% of the Lindane occurring in this tissue at any one time. All other tissues have the largest proportion of Lindane after the first day, and by Day 3 the proportions had fallen considerably. This corresponds with a proportional increase in the hepatopancreas. After day 3 a slight decrease in the proportion of Lindane in the hepatopancreas occurs, but it remains fairly constant. That of the gills also remains constant, whilst the proportion in the alimentary tract is seen to increase considerably. That of the cuticle, muscle, and gonads also increases slightly but levels off to a constant proportion.

Examination of the actual amounts ($\mu g.g^{-1}$) of Lindane in the tissues (Fig. 4.7) shows that although the relative proportion of toxicant was high on Day 1, the actual amounts in each tissue had not reached their maximum level. They continued to inrease, in the case of most tissues, until day 6. The concentration in the gills reached a maximum on day 3, and thereafter a significant reduction in the amount of Lindane occurred (Table 4.12, $P < 0.01$). No significant trend was observed for all other tissues ($P > 0.05$), a gradual increase in tissue concentrations being followed by a gradual decrease, showing no significant effect with time.

A decrease in the tissue Lindane concentrations probably would have occurred for some tissues (e.g. the gills) even if the external concentration had been kept constant. However, as illustrated in Fig. 4.6, the environmental levels of Lindane decreased considerably during the experiment, and so depuration of toxicant was probably able to occur at similar, or greater, rates than its uptake. Hence the decrease in the actual levels of Lindane observed in all tissues.

Fig. 4.9 shows the results expressed in Table 4.13 for the concentration factor. It reveals that although a gradual increase followed by a decrease in the actual concentrations of toxicant in the tissues occurred, the levels relative to those in the environment did not show this pattern. The gills accumulated the maximum amount of Lindane by Day 3, and consistent with the previous results, maintained lower levels thereafter. The trend with time was significant ($P > 0.05$),. All other tissues are seen to accumulate Lindane with increasing time of exposure up to Day 9, but a decrease is apparent by Day 12. The results were significant for both the cuticle and alimentary tract ($P < 0.05$), but not for the other tissues. The level in the hepatopancreas was seen to increase dramatically up to Day 3, thereafter becoming almost constant. Thus the result that there was no significant effect with time is in itself significant, indicating that the concentration factor for the hepatopancreas remains stable with increasing time of exposure. This indicates that some regulation of the tissue concentration may be possible, but whether or not the same result would have been achieved with constant external conditions would depend upon the capacity of the hepatopancreas

to eliminate Lindane.

4.2(iii) DISCUSSION

The results presented in this study, although primarily designed to show the accumulation of Lindane by tissues of *A.pallipes*, also showed that elimination occurs. Lindane did not become 'fixed' in the tissues and some translocation apparently occurred. Removal of Lindane from the environment was also demonstrated.

Accumulation of pesticides can occur from the aquatic environment, from food, or from sediments. Uptake of PCB's, pesticide-like substances, occurs from sedmiments into the tissues of shrimps, *Palaemonetes pugio* (Nimmo *et. al.*, 1974) and uptake from the natural aquatic environment polluted by organochlorine pesticides has been demonstrated in both marine (e.g., Albright *et. al.*, 1975) and freshwater (e.g. Diamond *et. al.*, 1968) crustaceans. Uptake into the tissues is much greater from the aquatic environment than from food for *Daphnia magna* and αHCH (e.g., Canton *et. al.*, 1975), whilst in fish, D.D.T. is seen to increase more from the food than water (Macek *et. al.*, 1979). Sieper (1972) reported that Lindane residues in fish were accumulated through the skin, gills and food. For crayfish accumulation would only be through the gills and diet due to the impermeable nature of the exoskeleton.

The organo-chlorine insecticides tend to accumulate more than other pesticides in the tissues of aquatic animals (relative to the amount in the environment) due to their greater solubility in lipids than water. Table 4.14 summarizes part of the literature which reports the uptake of organochlorine insecticides by crustaceans, and the uptake of PCB's is also shown for a marine and freshwater crustacean. Absorption and elimination of insecticides

by fish is reported to occur simultaneously, and most accumulation occurs in the tissues with the highest lipid content. However, it is suggested that in the short term, accumulation involves factors other than simply the liphophilicity of the insecticide, and fat contents of the tissues (Kahn, 1977). The total lipid contents of various tissues were estimated in this study by the method of Bligh and Dyer (1959) and it was shown that the tissues with the highest lipid content were: the hepatopancreas (males, 43.13%; females, 44.23%) > Gills (M, 27.68%; F, 26.99%) > alimentary tract (M, 25.12%; F, 26.34%) > muscle (M, 9.80%; F, 10.11%) > cuticle (M, 7.36%; F, 9.05%). Results were based on the pooled tissues of 5 animals (to gain sufficient material), and the lipid contents appear high since the proportions relate to the dry tissue. The gonads are not included in the above rank-order; they were the only tissues to reveal a sex difference (M, 26.99%; F, 45.93%). Lipid content has been shown to be an important factor in the tissue accumulation of not only insecticides, but also herbicides such as Atrazine (e.g., Streit, 1978).

This study reported that no differences occurred between male and female crayfish for the uptake of Lindane into any of the tissues. This is perhaps surprising in view of the higher fat content of the female ovary than the male testes. Airaksinen *et. al.*,(1976) reported that female *Astacus astacus* accumulate more D.D.T. than males due to their higher lipid content, and lobsters (*Homarus americanus*) caught in natural waters polluted with D.D.T. are found to accumulate more insecticide in the egg mass, in the case of females, than in the hepatopancreas (Guarino *et. al.*,1974).

The order of accumulation in the tissues after 7 days was reported, and that over 12 days was seen to vary slightly from the situation on Day 7. The hepatopancreas, however, consistently accumulated more than the other tissues. This was also the case for D.D.T. in the shrimp - *Penaeus duorarum* (Nimmo *et. al.*, 1970), and lobster - *Homarus americanus* (Guarino *et. al.*, 1974). In the latter study labelled D.D.T. was injected into the blood system, applied in the diet, and applied in the aquatic environment. In each case the toxicant was rapidly removed to the hepatopancreas, which accumulated 90% of the total body burden, reaching a maximum after 7 days. The proportion found to be in the hepatopancreas in the present study varied between 60% and 90% and reached a maximum after 3 days. Guarino *et. al.*, (1974) reported high fat contents for the hepatopancreas, as observed in this study, and this may partly explain the high levels of insecticide found in the organ.

Physiologically, its two principle functions are absorption and storage of nutritive products, and this ability may also explain the capacity of the hepatopancreas to accumulate insecticides. In this study Lindane was seen to accumulate as much as 39 times over that in the environment. Within the hepatopancreas metabolism of the Lindane may occur to its breakdown products. Hence the radioactivity recorded may not indicate the presence of Lindane, but a metabolite. However, the lack of mixed function oxidases in the hepatopancreas of crayfish such as *Astacus astacus* has been discussed (3(iv)) and so it is probable that the radioactivity recorded, relates to Lindane itself. Indeed it was suggested that the lack of a suitable detoxification mechanism

might explain the high degree of susceptibility of crustaceans to Lindane (3(iv)). That the hepatopancreas is able to accumulate Lindane preferentially, however, is in itself a detoxification method, since the amount of toxicant available to act on the nervous system is reduced (Lindane being a neuro-toxin). Additionally the results of experiment 2, which indicate that high initial levels of Lindane occur in all tissues, followed by elimination apparently to the hepatopancreas, may explain why the loss of balance reported previously (3, see also 5(iv)), is regained with continued exposure to toxicant.

Experiment 2 effectively showed both accumulation and elimination of Lindane by the tissues of *A. pallipes*. It was not possible to examine a range of toxicant concentrations, and neither was it possible to maintain the single concentration at a constant level. Hence similar results were not achieved as in 4.1. Other authors have reported that the uptake of pesticides increases with increasing external concentration and also with time of exposure (e.g. Schimmel *et. al.*, 1977a, 1977b), although with the herbicide Atrazine the relationship was not linear, but was shown to be hyperbolic with both time and increasing external concentration (Streit, 1978). When the concentration factor was considered, a decrease in the accumulation in the tissues relative to that in the environment was seen to occur as the external concentration increased, for: marine shrimps - *Palaemonetes pugio*, *Penaeus duorarum* exposed to Lindane (Schimmel *et. al.*, 1977a), Marine shrimp - *P. duorarum* exposed to Toxaphene (Schimmel *et. al.*, 1977b), and various aquatic organisms exposed to Atrazine (Streit, 1978). The concentration factor was observed

to increase with increasing time of exposure to a single concentration of toxicant for crayfish exposed to PCBs (Mayer *et.al.* 1977). This study reported a gradual increase in concentration factor in the hepatopancreas with time, but the result was not significant, although a significant increase in the cuticle did occur with time. The highest concentration factor was found when the level of Lindane in the beakers was at its lowest. Most concentration factors reported in the literature, relate to the whole animal and not to individual tissues. Compared to the other freshwater crustaceans reported in Table 4.14, the concentration factor for *A. pallipes* (this study) is of the same order as that for *Daphnia magna* exposed to 0.01 p.p.m. α HCH, although the time scale was greater (Canton *et. al.*, 1975). Crayfish exposed to PCBs tended to accumulate considerably more toxicant (Mayer *et. al.* 1977). The marine crustaceans also accumulated more insecticide than the freshwater crustaceans for similar environmental concentrations, and it was also true that PCBs tended to be concentrated to a greater degree than the insecticides (e.g., PCBs - Nimmo *et. al.*, 1977a; 1977b).

Elimination of pesticides on transferring an organism to clean water occurs at a rate dependent upon the water solubility of the insecticide, and for fish, all organs eliminate residues at about the same rate (Kahn, 1977). About 50% of the D.D.T. in the hepatopancreas of marine shrimps was lost in 5 days upon removal of the animals to clean water (Nimmo *et. al.*, 1970), and in 46 days for lobsters (Guarino *et. al.*,1974). Schimmel *et.al.*(1977a) have reported that shrimps previously exposed to Lindane are capable of eliminating all of the toxicant from their tissues

in clean water. That *A. pallipes* is also capable of eliminating Lindane is apparent from the reduction in actual levels of toxicant in the tissues with time. The increasing amount in the alimentary tract, as observed both as actual amounts, and as a proportion of the total Lindane, indicates that elimination may be occurring via this route. The gills were shown to remove Lindane from their tissues and this was presumably an active process. It is possible that Lindane was being transferred to the hepatopancreas, and indeed on Day 3 an increase in the proportion of Lindane in the hepatopancreas was accompanied by a decrease in all other tissues. However, it is also possible that the gills themselves may also eliminate Lindane directly into the environment. The observation that Lindane concentrations in the beakers levelled off to a plateau with time may in part have been due to the fact that Lindane was being returned to the environment at similar rates to its removal.

From the above discussion it would thus appear that Lindane uptake occurs through the gut via the oral route, and through the gills. Initially uptake may not be active but simply the passage of the liphophilic insecticide into the fats of the tissues. However, that active processes also operate seems apparent from the way in which toxicant levels in various tissues are regulated. Those in the gills are kept to a low level after an initial rapid uptake, during which time the active processes may have been stimulated. The increase in the alimentary tract also seems to indicate active removal of Lindane otherwise one should have expected it to remain in the Lipid rich hepatopancreas. The levelling off of the concentration factor result in the hepatopancreas

is related to a decrease in the actual amount of Lindane in that tissue, and may indicate that elimination is actively occurring, and that an equilibrium has been reached between continued uptake and removal. The results certainly show that upon brief exposure to an accidental flush of pollutant, *A. pallipes* would be able to regulate the amounts of Lindane in the tissues, and hopefully survive. Also exposure to sublethal levels may be tolerated by adults since effective detoxification occurs by the storage of Lindane in the hepatopancreas. Problems of saturation of the tissues should not arise, since it seems that an equilibrium between uptake and removal of the insecticide can be reached. These results show that accumulation of Lindane can occur from what were previously described as 'safe concentrations' (3(iv)).

TABLE 4.10 THE UPTAKE OF ^{14}C. LINDANE BY MALE AND FEMALE CRAYFISH

TISSUE TYPE	TISSUE ^{14}C.LINDANE CONTENT, c.p.m. mg^{-1} WET WEIGHT						COMPARISON OF THE SEXES		
	MALES			FEMALES					
	RANGE	MEAN	S.E.	RANGE	MEAN	S.E.	t	df	P
Cuticle	17.81-23.40	20.61	2.80	11.53-34.73	22.50	6.73	0.21	4	>0.1
Gills	33.06-44.60	38.83	5.77	21.27-60.32	46.44	12.61	0.45	4	>0.1
Hepatopancreas	71.75-186.86	134.22	33.59	45.75-184.10	125.51	41.32	0.17	4	>0.1
Alimentary tract	77.98-97.93	89.36	5.93	23.22-84.29	63.66	20.22	1.22	4	>0.1
Gonads	30.76-62.31	43.35	9.50	19.98-59.10	41.37	11.44	0.17	4	>0.1
Tail muscle	14.41-29.41	18.89	5.28	10.02-37.30	23.96	7.88	0.53	4	>0.1

TABLE 4.11 TO SHOW THE PERCENTAGE CUMULATIVE LOSS OF ^{14}C. LINDANE FROM THE WATER CONTAINING THE CRAYFISH (±1 S.D.)

DAY	BEAKERS + ANIMALS		CONTROL (1 ONLY)	EXPERIMENT
	MEAN	S.D.		
1	33.68	11.41	2.94	1
2	44.92	14.17	11.31	
3	56.12	10.70	12.48	
4	64.17	9.20	17.79	
5	68.31	8.57	35.24	
6	70.35	8.32	49.72	
7	71.26	8.38	62.77	
1	15.41	6.56	13.57	2
3	48.59	13.39	9.16	
6	59.56	5.73	26.13	
9	66.75	6.14	32.53	
12	68.23	7.79	37.78	

TABLE 4.12 UPTAKE OF ^{14}C. LINDANE BY TISSUES OF *A. PALLIPES* OVER 12 DAYS

TISSUE TYPE	TIME (DAYS)	TISSUE ^{14}C. LINDANE CONTENT				1 WAY ANOVAR (TIME)		
		(i) As $\mu g g^{-1}$ wet weight			(ii) PROPORTION OF TOTAL (%)			
		RANGE	MEAN	S.E.		F	df	P
CUTICLE	1	0.04-0.08	0.0625	0.0110	10.22	1.89	4,10	0.188
	3	0.08-0.09	0.0823	0.0031	2.18			
	6	0.06-0.12	0.0827	0.0209	6.11			
	9	0.03-0.06	0.0492	0.0100	5.43			
	12	0.04-0.06	0.0470	0.0104	6.23			
GILLS	1	0.05-0.08	0.0710	0.0106	7.83	6.16	4,10	0.009
	3	0.13-0.35	0.2519	0.0664	4.31			
	6	0.08-0.13	0.1042	0.0139	4.04			
	9	0.05-0.09	0.0739	0.0148	4.21			
	12	0.05-0.08	0.0673	0.0085	5.85			
HEPATO-PANCREAS	1	0.13-0.31	0.1948	0.0565	58.86	1.61	4,10	0.056
	3	0.41-1.26	0.7443	0.2636	87.95			
	6	0.32-1.19	0.6898	0.2601	72.45			
	9	0.31-0.53	0.3859	0.0698	69.59			
	12	0.21-0.67	0.3988	0.1395	76.05			
ALIMENTARY TRACT	1	0.06-0.09	0.0701	0.0111	8.42	3.32	4,10	0.056
	3	0.16-0.23	0.1910	0.0210	2.97			
	6	0.19-0.47	0.2861	0.0939	9.24			
	9	0.13-0.21	0.1732	0.0230	11.22			
	12	0.08-0.15	0.1078	0.0217	6.97			
GONADS	1	0.06-0.11	0.0879	0.0172	4.23	1.57	4,10	0.257
	3	0.07-0.13	0.1002	0.0181	0.40			
	6	0.08-0.17	0.1298	0.0259	1.49			
	9	0.05-0.16	0.1145	0.0338	3.95			
	12	0.02-0.07	0.0550	0.0136	1.85			
TAIL MUSCLE	1	0.03-0.04	0.0386	0.0027	10.43	1.49	4,10	0.276
	3	0.03-0.07	0.0535	0.0107	2.17			
	6	0.04-0.09	0.0635	0.0158	6.66			
	9	0.02-0.06	0.0437	0.0134	6.42			
	12	0.02-0.04	0.0289	0.0070	5.39			

TABLE 4.13 TO SHOW THE UPTAKE OF ^{14}C. LINDANE OVER 12 DAYS, EXPRESSED AS A CONCENTRATION FACTOR

TISSUE TYPE	TIME (DAYS)	CONCENTRATION FACTOR			1 WAY ANOVAR (TIME)		
		RANGE	MEAN	S.E.	F	df	P
CUTICLE	1	1.40-2.18	1.84	0.23	3.62	4,10	0.045
	3	2.87-4.54	3.79	0.49			
	6	3.17-6.00	4.42	0.83			
	9	3.05-4.74	4.04	0.51			
	12	3.25-5.15	4.46	0.61			
GILLS	1	1.53-2.63	2.13	0.32	3.95	4,10	0.036
	3	4.68-14.12	12.07	3.82			
	6	4.11-6.62	5.66	0.78			
	9	4.44-7.63	6.10	0.92			
	12	6.24-6.55	6.51	0.14			
HEPATO-PANCREAS	1	3.86-9.98	5.99	0.20	1.94	4,10	0.179
	3	20.80-62.20	34.66	13.77			
	6	20.97-57.42	35.81	11.05			
	9	29.94-34.56	31.67	1.45			
	12	18.44-59.32	38.82	11.80			
ALIMENTARY TRACT	1	1.81-2.53	2.09	0.22	5.15	4,10	0.016
	3	6.66-11.89	8.82	1.58			
	6	9.69-22.81	15.16	3.94			
	9	11.76-19.56	14.72	2.44			
	12	7.13-13.33	10.67	1.84			
GONADS	1	1.54-3.49	2.65	0.58	3.19	4,10	0.062
	3	3.50-4.94	4.45	0.47			
	6	5.30-8.19	6.90	0.85			
	9	4.72-14.83	9.51	2.93			
	12	3.48-6.28	5.16	0.85			
TAIL MUSCLE	1	1.10-1.19	1.15	0.03	2.45	4,10	0.114
	3	1.67-2.86	2.38	0.36			
	6	2.20-4.54	3.42	0.68			
	9	1.68-5.41	3.59	1.08			
	12	1.86-3.17	2.72	0.43			

TABLE 4.14 THE UPTAKE OF PESTICIDES AND PCB'S BY AQUATIC CRUSTACEANS

ORGANISM	COMPOUND	CONCENTRATION (p.p.m.)	EXPOSURE TIME	TISSUES AFFECTED	CONCENTRATION FACTOR (C.F.)	NOTES	REFERENCE
FRESHWATER:							
A. pallipes	LINDANE	0.03	7 days	Hp>>A.T.>G/G_N>C>M		Male + female, no sig. dif.	Author
		0.034	1-12 days	Hp>>rest, varies	38.82	Max. C.F., Hp Day 12	
Daphnia magna	α-HCH	0.01-0.80	3-96 hrs.	Whole body	60-350	Uptake from H_2O>food	Canton *et. al.*,1975
'crayfish'	PCB's	0.0012	1-21 days	Whole body	160-750	C.F. increases with time	Mayer *et.al.*, 1977
MARINE:							
Palaemonetes pugio	LINDANE	0.001-0.0055	96 hrs.	Whole body	25-80	C.F. decreases as Ext. conc.inc.	Schimmel *et.al.*,1977a
Penaeus duorarum	LINDANE	0.00003-0.00062	96 hrs.	Whole body	32-143	C.F. dec. as ext. conc. inc.	
	BHC	0.00019-0.00068	96 hrs.	Whole body	56-142	C.F. dec. as ext. conc. inc.	
P. duorarum	TOXAPHENE	0.0006-0.004		Whole body	400-600	C.F. dec. as ext. conc. inc.	Schimmel *et.al.*,1977b
P. pugio	TOXAPHENE	0.0034-0.0083		Whole body	800-1,200	C.F. inc. as ext conc. inc.	
P. pugio	PCB's	0.00017-0.0091	7 days	Whole body	3,000-11,000		Nimmo *et.al.*, 1974
P. duorarum	D.D.T.	0.00005-0.0002	1-56 days	Hp>>N>H>A.T.>G>C>M	14,000-30,000	C.F. in Hp	Nimmo *et.al.*, 1970
Homarus americanus	^{14}C.D.D.T.	Injected	-	Hp>>A.T.>N>G_N>G>M		90% D.D.T. in Hp	Guarino *et.al.*, 1974
		Environmental	7 days	Hp>>A.T.>G_N>G/M>B>N		'Natural' levels Eg>Hp	

FIG. 4.5 THE QUENCH-CURVE USED TO CORRECT ALL RESULTS IN 4.2
(THE LINE WAS FITTED BY REGRESSION, r = 0.9901)

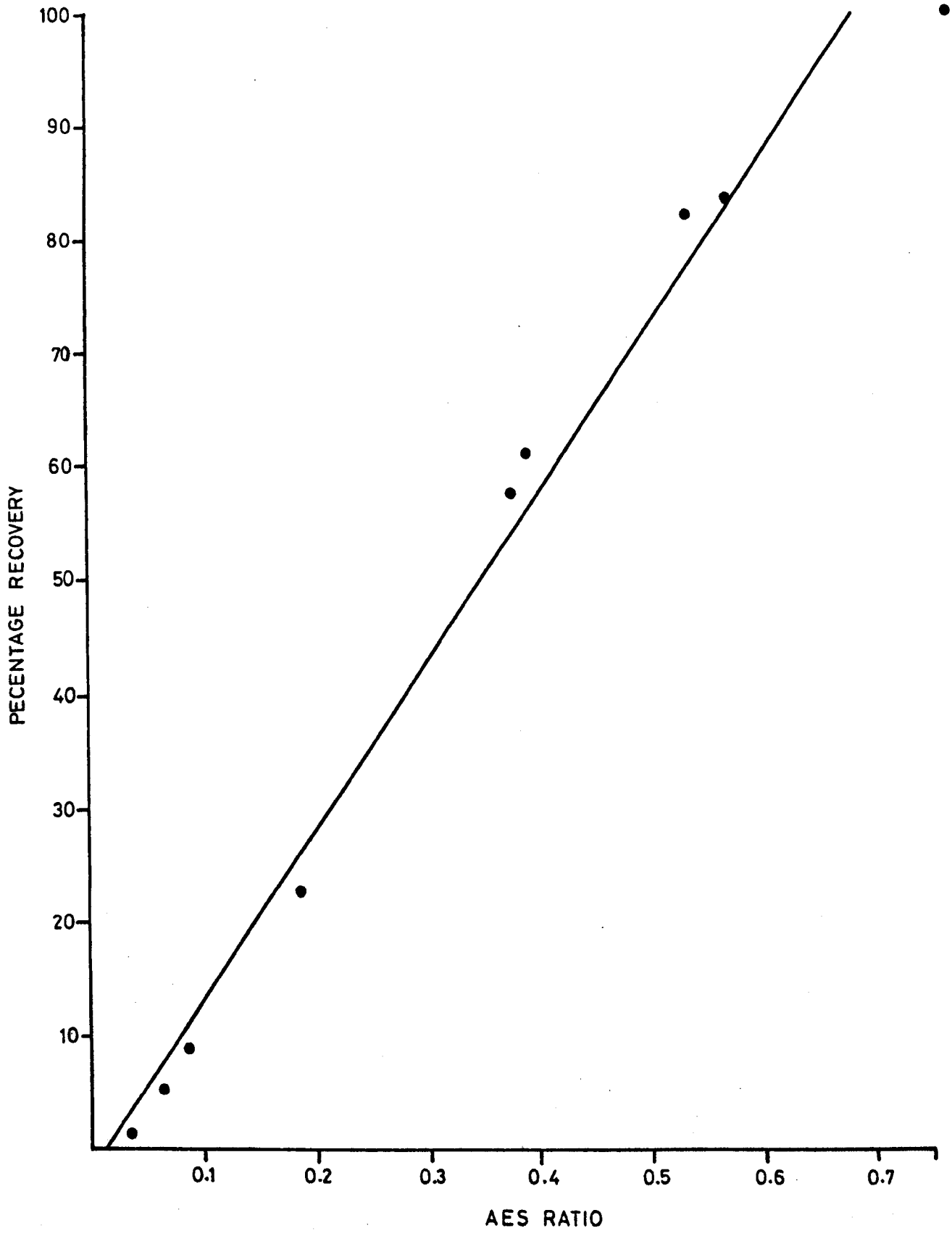
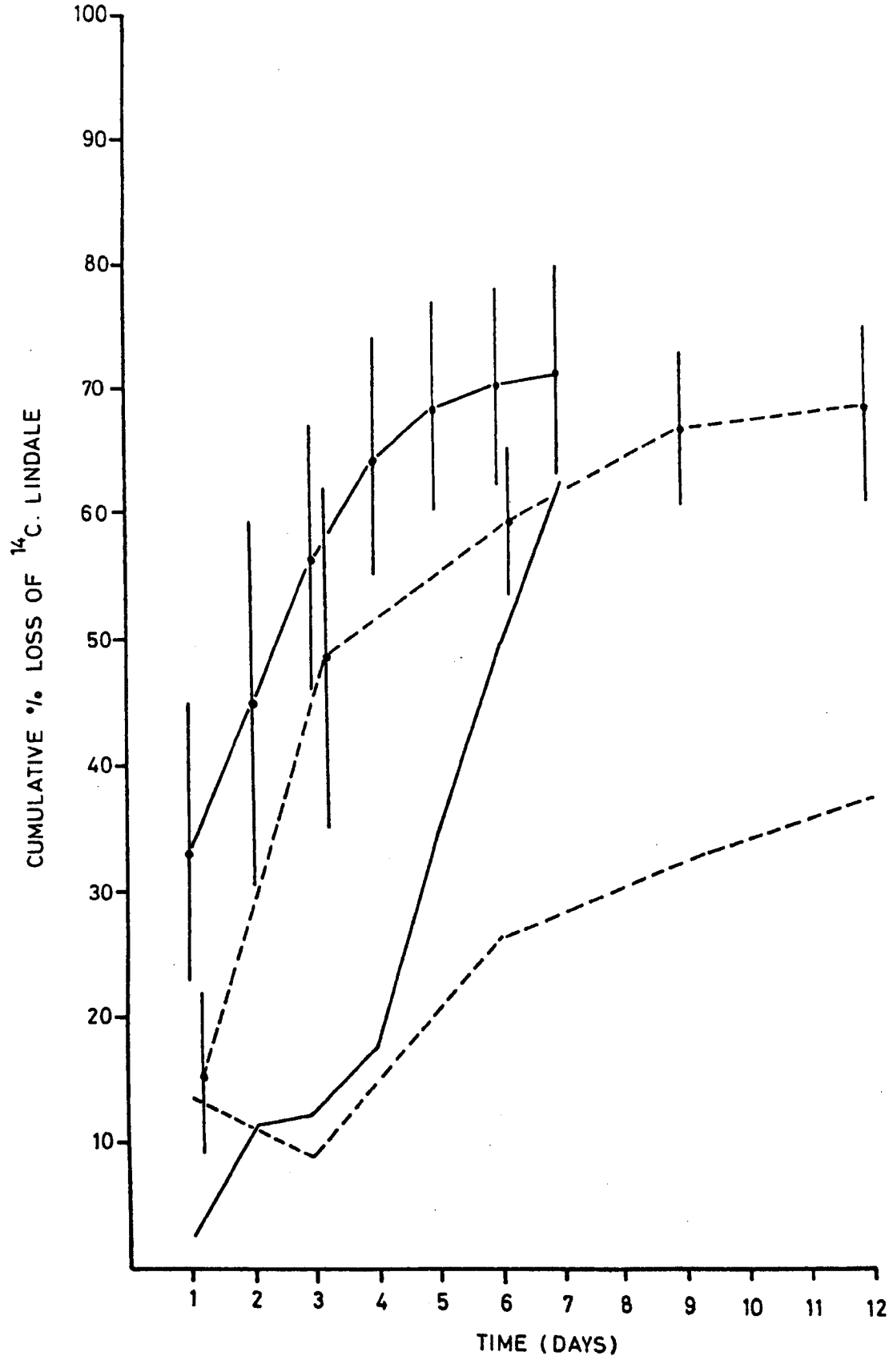


FIG. 4.6 TO SHOW THE LOSS OF ^{14}C LINDANE FROM BEAKERS WITH ANIMALS (±1 S.D.) AND CONTROLS WITH NO ANIMALS (WITHOUT S.D. BARS). (RESULTS FOR BOTH EXPERIMENT 1(——), AND 2 (---) ARE SHOWN)



FIGS. 4.7 - 4.9 THE UPTAKE OF ^{14}C. LINDANE OVER 12 DAYS

These figures show the uptake of Lindane by animals environmentally exposed to 0.034 p.p.m. total Lindane (0.02 p.p.m. cold, 0.014 p.p.m. hot). The results are given for the following tissues: Cuticle (CUT.), Gills (GIL.), Hepatopancreas (Hp.), alimentary tract (A.T.), gonads (GON.) and tail muscle (MUS.).

Figs. 4.7a and b show the results expressed as $\mu g\ ^{14}C$. Lindane g^{-1} wet weight of tissues, ± 1 S.E.

Fig. 4.8 shows the results expressed as a proportion of the total Lindane occurring in each tissue.

Fig. 4.9 shows the results expressed as a concentration factor ± 1 S.E.

In each case accumulation is expressed against time of exposure to toxicant (Days).

Fig. 4.7a

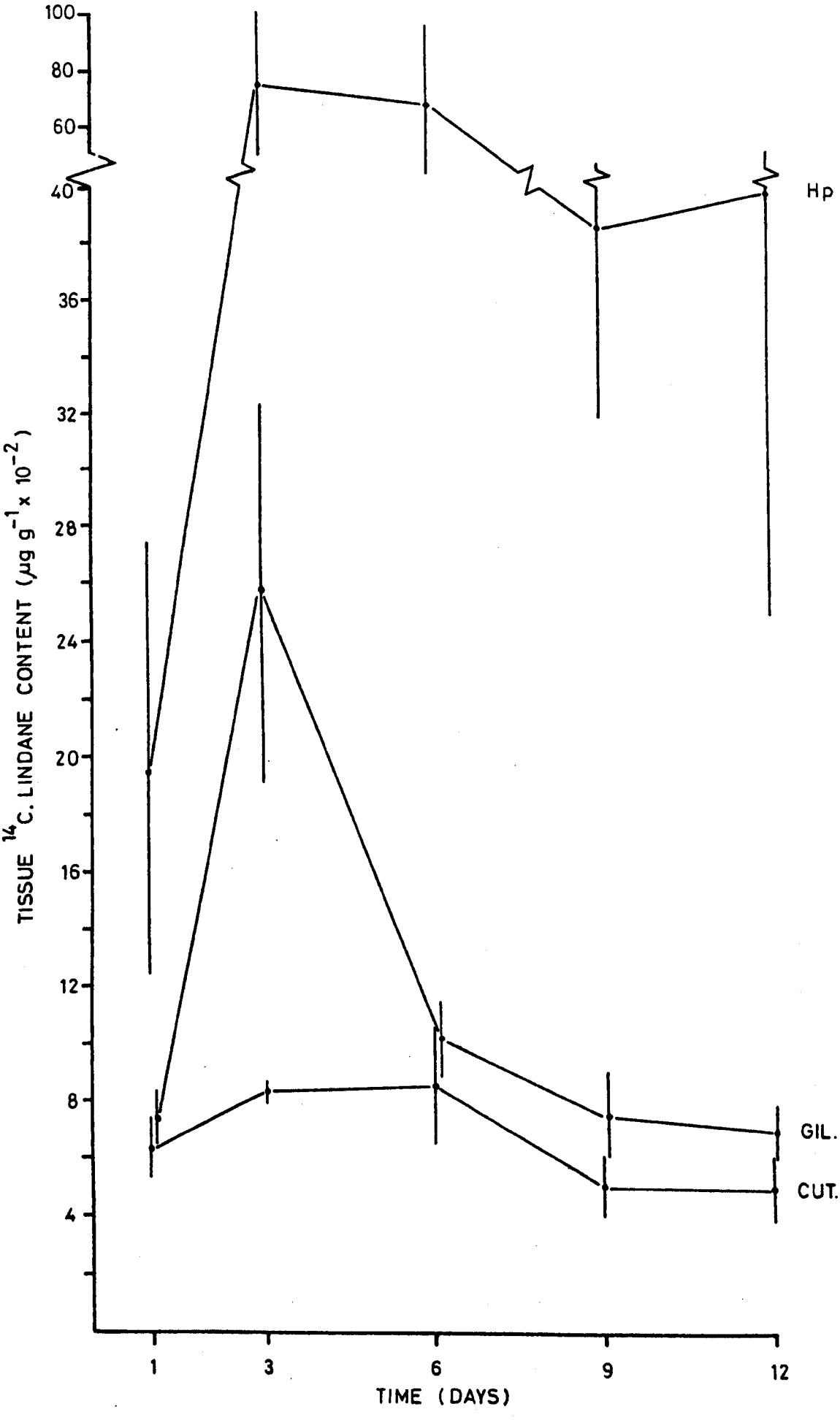

100
80
60
40
36
32
28
24
20
16
12
8
4
TISSUE ^{14}C. LINDANE CONTENT ($\mu g\ g^{-1} \times 10^{-2}$)
Hp
GIL.
CUT.
1
3
6
9
12
TIME (DAYS)

Fig. 4.7b

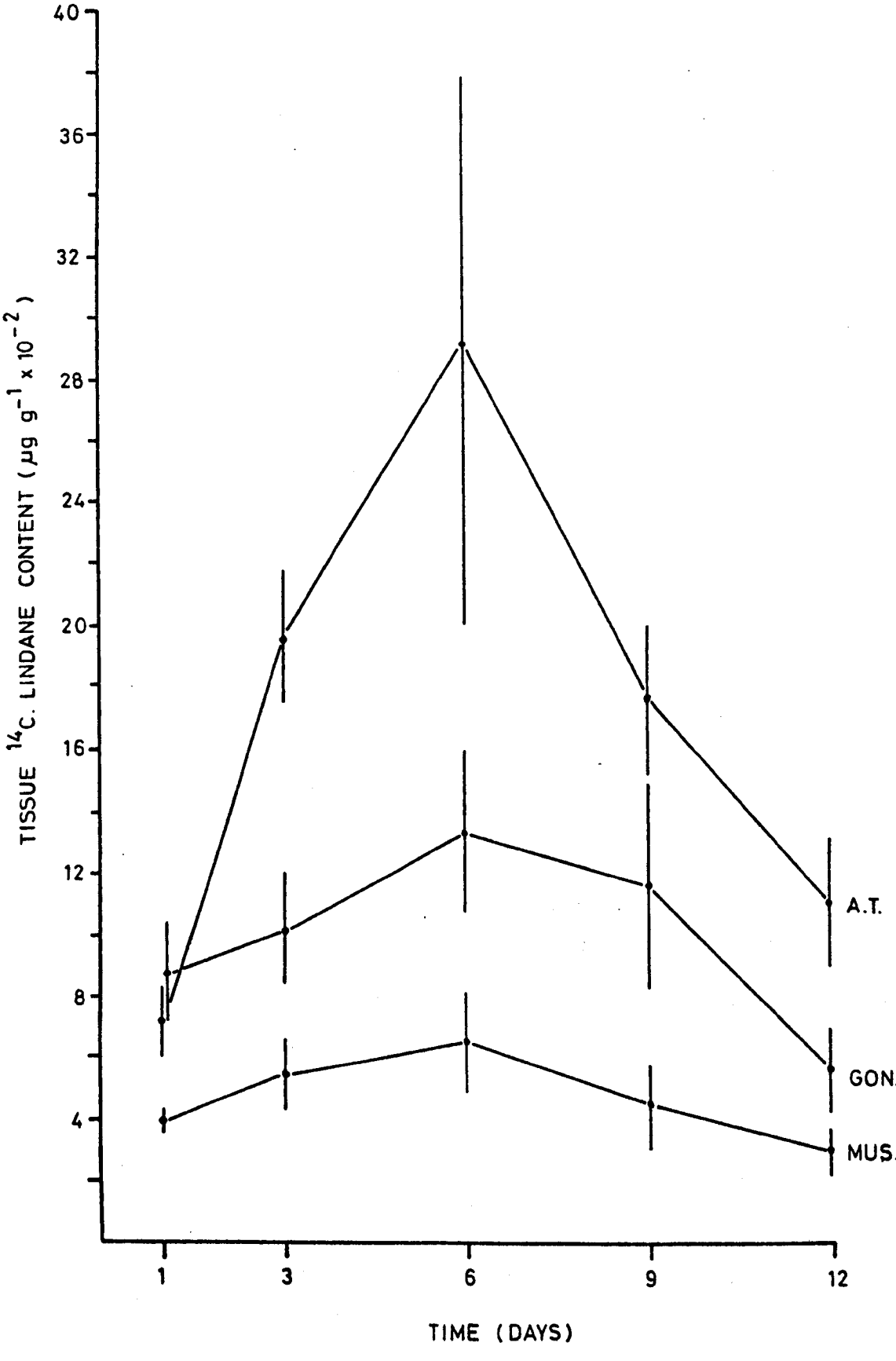

40
36
32
28
24
20
16
12
8
4
TISSUE ^{14}C. LINDANE CONTENT (µg g^{-1} x 10^{-2})
1
3
6
9
12
TIME (DAYS)
A.T.
GON.
MUS.

Fig. 4.8

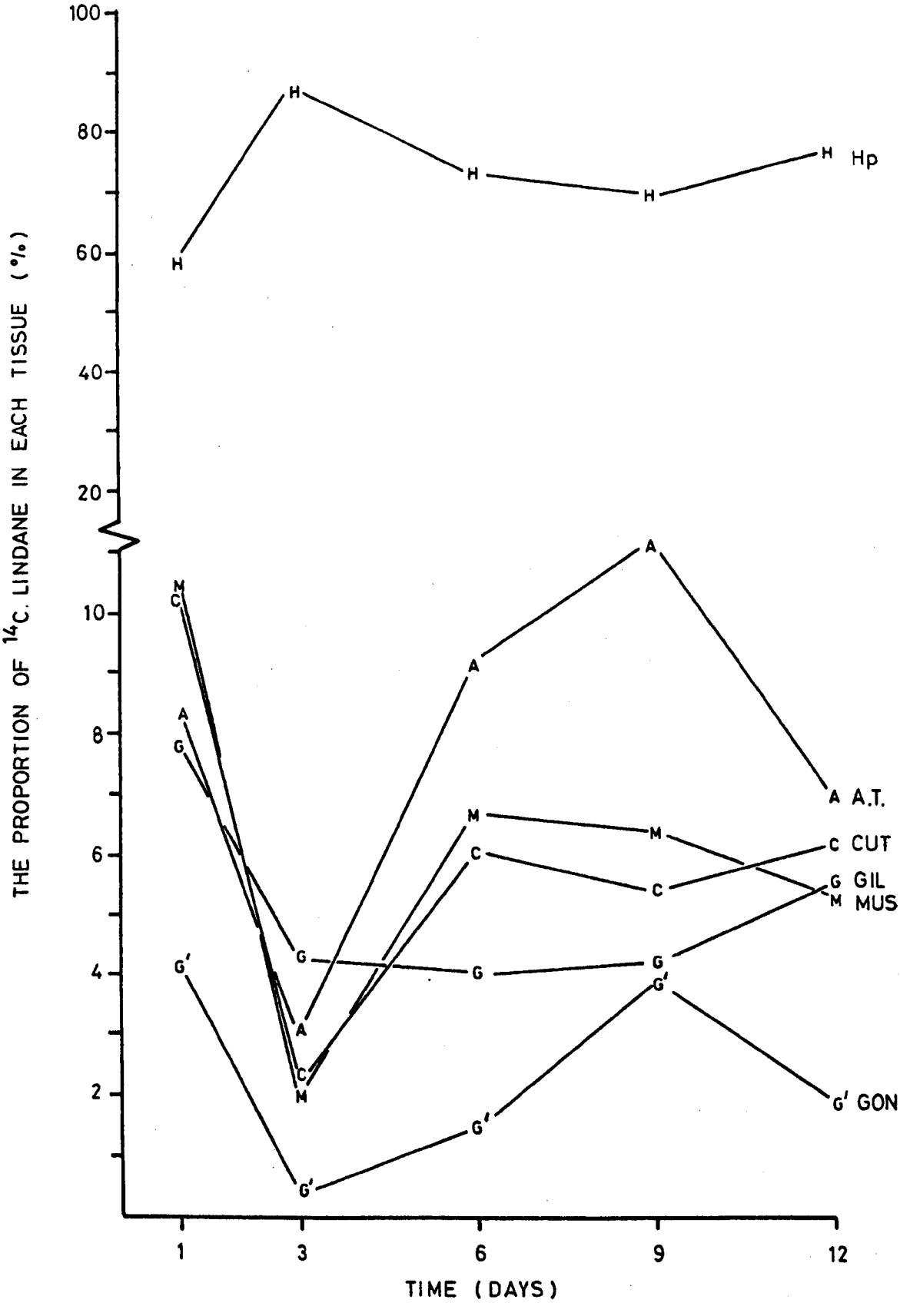
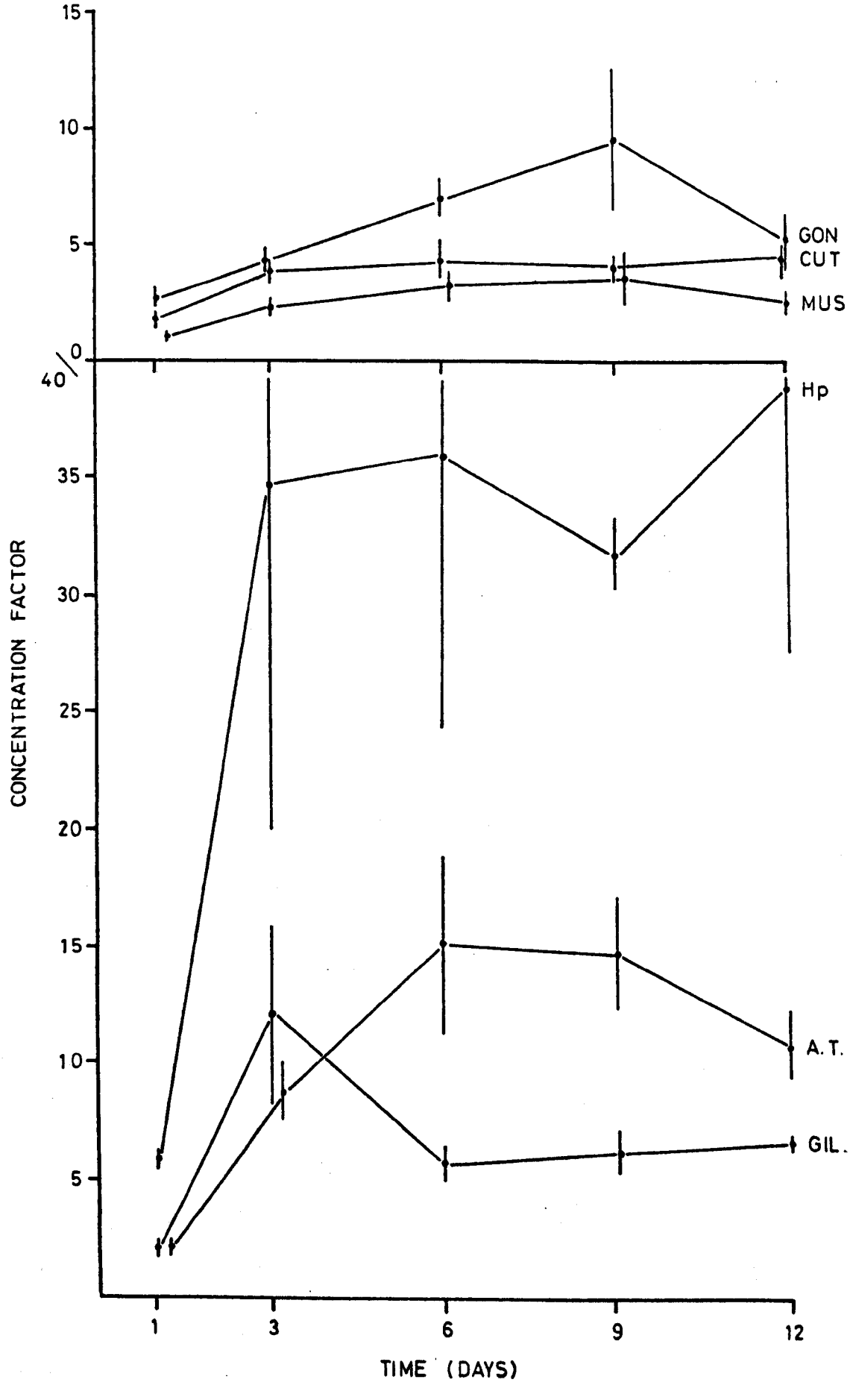

THE PROPORTION OF ^{14}C. LINDANE IN EACH TISSUE (%)
100
80
60
40
20
10
8
6
4
2
1
3
6
9
12
TIME (DAYS)
H Hp
A A.T.
C CUT
G GIL
M MUS
G' GON

Fig. 4.9


15
10
5
0
40
35
30
25
20
15
10
5
GON
CUT
MUS
Hp
A.T.
GIL.
CONCENTRATION FACTOR
1
3
6
9
12
TIME (DAYS)

CHAPTER 5

THE EFFECTS OF CADMIUM AND LINDANE UPON RESPIRATION IN *A. PALLIPES*

5(i) INTRODUCTION

Acute toxicity tests are able to provide information relating to the lethal effects of potential pollutants but the results are not always directly transferable to the field situation. Chronic studies, however, which employ low concentrations of toxicant and study their effect over a longer time period relate more closely to the situation encountered in the field. Respiration and oxygen uptake is a very sensitive monitor of the effects of pollution and provides an ideal system with which to study chronic toxicity. It is useful since energy processes requiring oxygen serve as an indicator of overall physiological state, and small changes may be detected so respiration rate becomes a very delicate physiological indicator of the levels of both oxidative processes and biochemical reactions occurring within the tissues of an organism (Andryuschenko, 1972; Sigmon, 1979).

Respiratory experiments have been conducted both *in vivo* and *in vitro*, and respirometers of a suitable size for crayfish have been described (e.g. Spencer Davies, 1966). Studies have also shown that no effect of pollutants may be detected *in vivo* (e.g. Collier *et. al.*, 1973; Anderson, 1978) whilst *in vitro* the rate of respiration is apparently altered (Collier *et. al.*1973). The explanation lies in the fact that the live animal compensates for pollutant induced changes in respiration rates, whilst this is not possible *in vitro*. Consequently although studies using the live animals will indicate the actual response to a pollutant which would be experienced in the field, studies

using excised gill tissues more accurately describe the physiological state of the animal. Thus, although studies *in vitro* would appear to be the unnatural choice, they do in fact provide more information.

Using excised gill tissue it should be possible to monitor the normal rate of respiration, add toxicant to the medium, and then record any changes observed. Such a technique, however, is entirely artificial and would not relate to the response of the organism as a whole. Furthermore, owing to the short time period of such a study, relatively high concentrations of toxicant would be required, and thus the object of studying chronic sublethal effects of pollutants could not be achieved. Instead, a method of exposing the live animals to low concentrations of toxicant for a certain time period was employed. Similar studies have been conducted using molluscs (e.g. Engel, 1979) and crustaceans (e.g. Thurberg *et. al.*, 1973; also 5(iv)). The excised tissues were then monitored in a Rank oxygen electrode in clean water. This has the additional advantage that no toxicant is in the respirometer which could potentially affect the results, particularly cadmium ions which may alter the electrical properties of the electrodes used. By this method the rates of respiration of excised gill filaments from *A. pallipes* were studied after exposure to various concentrations of cadmium and Lindane, and the results are now presented.

5(ii) MATERIALS AND METHODS

Markfield Quarry stock obtained during 1981 were subject to the usual pre-experimental procedure (2) and were used for all of the experiments conducted in this section. They were acclimatized to a temperature of 15 ±1°C, and intermoult animals

of both sexes were used at a ratio of 2:1, males:females within each trial. Their sizes varied between 30 mm and 40 mm carapace length ($\bar{x}$ ± S.D. = 34.49 ± 3.48 mm). Experiments were conducted at 15°C in a static water system as described in 2, except that at the risk of volatilization of the toxicant, aeration was employed owing to the relatively long term nature of the experiment. Cadmium and Lindane were added as required, except in the control tanks, and throughout the experimental period the pH of the water varied between 7.25 and 7.65.

The effect of toxicant upon the oxygen consumption of excised gill tissue was examined after exposure of the intact animal to toxicant with both increasing toxicant concentration over a fixed time period, and increasing time of exposure with a fixed concentration of toxicant. The following regimes were conducted;

CADMIUM

(i) 1 p.p.m. and 10 p.p.m. Oxygen consumption was monitored after exposure to toxicant on each of days 1, 3, 6 and 9.

(ii) 1 p.p.m., 5 p.p.m., 10 p.p.m. 25 p.p.m. 50 p.p.m. Oxygen consumption was monitored after exposure to the toxicant for 3 days.

N.B. Toxicant concentrations were chosen after initial toxicity tests using Nanpantan stock indicated that *A. pallipes* was tolerant to high levels of cadmium (see 3). However, the Markfield stock were unable to tolerate the highest concentrations employed in this experiment, and so for the longer term studies, concentrations of 1 p.p.m. and 10 p.p.m. were chosen. In Chapter 3 it was concluded that 3.52 p.p.m. cadmium ions constituted a safe level of toxicant to adult Markfield

Quarry stock.

LINDANE

(i) 0.02 p.p.m. Oxygen consumption was monitored after exposure to toxicant on each of days 1, 3, 6 and 9.

(ii) 0.02 p.p.m., 0.05 p.p.m., 0.15 p.p.m., 0.20 p.p.m. Oxygen consumption was monitored after exposure to toxicant for 24 hours.

(iii) 0.01 p.p.m. and 0.03 p.p.m. Oxygen consumption was monitored after exposure to toxicant on each of days 3, 6, 9, 12 and in the case of 0.03 p.p.m. day 15 also (to check the day 12 result).

N.B. LD_{50} 96 hr. values for adult Nanpantan stock were 0.44-0.48 p.p.m. Lindane, with a safe concentration being 0.04 p.p.m. However, after 24 hours exposure at 0.20 p.p.m. all the Markfield Quarry stock were alive, but exhibiting loss of balance, and falling on their 'backs'. Hence the experiment was conducted on day 1, not day 3 as in the case of cadmium. (Note that this reinforces the observation made in 3 that the Markfield Quarry stock are more susceptible to pollutants than the Nanpantan stock. Alternatively the higher temperatures used in this study may provide the explanation).

The result of the longer term experiment at 0.02 p.p.m. appeared to show no differences in oxygen consumption from the control. Hence trial (iii) was instigated using 0.01 p.p.m. and 0.03 p.p.m. Lindane in order to check this result.

CONTROLS

Appropriate controls for each trial conducted were treated identically to the animals exposed to toxicant, excepting the

presence of toxicant (Acetone was used with those for comparison with the Lindane trials, see 2). Owing to the shortage of available equipment which limited the number of trials that could be conducted in any one day, the experimental design was restricted. Hence in the experiments involving exposure of *A. pallipes* to a range of toxicants, not all animals could be examined on the same day in reality, although they were examined on the same day in terms of exposure to toxicant. Separate controls were thus necessary for each individual day of trials, and this explains why in these experiments it was not possible to give a single value for the oxygen consumption of control animals against which to compare treated animals. All results are converted to S.T.P. which allows for daily variations in external environmental conditions.

EXPERIMENTAL PROCEDURE

The gills of three animals were used at each concentration and each time of exposure studied. The animals were exposed to toxicant of the required concentration and for the appropriate time period, and then removed to clean water for approximately two hours prior to use in the respirometry experiments. This was in order to allow the toxicant to be flushed out of the branchial chambers and from the external surfaces of the gill filaments in case the presence of toxicant would affect the electrodes of the respirometer. The gills were then dissected out and all of the gill filaments from the left hand side of the animal were placed in one respirometer, and those from the right in another. The rates of oxygen consumption (respiration) of the excised gill tissue were measured using a Rank oxygen electrode, the principles of operation of which are given in Fig. 5.1. The results

were recorded on a JJ chart recorder (Model CR 100) which was connected to the electrode.

Each experiment was conducted in 3 mls of air saturated stock-tank water maintained at 15°C ±1°C by means of a circulating water system employing a heated water bath and cooling coil to maintain the temperature. Prior to conducting measurements using excised gill tissue it was necessary to calibrate the electrode. The procedure is illustrated in Fig. 5.2. Calibration was conducted after every other experiment in case of any changes in the sensitivity of the electrode, and the barometric pressure each day was recorded to enable calculation of the results as a volume of gas at S.T.P.

Excised gill tissue placed directly into 3 mls of air saturated water at 15°C in the respirometer was allowed to 'recover' and equilibrate for 30 minutes before any readings were taken. After the equilibration period air was bubbled into the water to bring the available oxygen level back up to 100%, and the experiment was started. Oxygen consumption was recorded after each of two 15 minute intervals. On completion of the experiment the gills were removed and dried (see 2), and after the weight had been recorded this same gill tissue was, in the case of the cadmium trials, analysed for the cadmium content (see 4.1). The results of the respirometry experiments are expressed as $\mu l\ O_2$ consumed at S.T.P. per mg dry weight of tissue per hour. Standardized thus, all results are directly comparable. The following is an example of the calculations applied to all the results.

(1) To calculate $\mu l\ O_2$ available in 3 ml of air saturated water at 15°C.

- Assume a temperature of 15°C and a barometric pressure of 761.4 mm of mercury. At 15°C for air saturated water the International Critical Tables give a value for K, the Henry's law constant, as:-

$$K = 2.766 \times 10^7$$

now, $K = \frac{P_{O2}}{x_{O2}}$ (Henry's law)

where P_{O2} = Partial pressure of oxygen

x_{O2} = mole fraction of oxygen

Air contains 21% oxygen,

$$\therefore P_{O2} = 0.21 \times 761.4$$

$$= 159.8 \text{ mm Hg.}$$

so, $$x_{O2} = \frac{P_{O2}}{K} = \frac{159.8}{2.766 \times 10^7}$$

$$= 5.78 \times 10^{-6} \text{ moles.}$$

Also, $$x_{O2} = \frac{N_{O2}}{N_{O2} + N_{H2O}}$$ (Raoult's law)

where, N_{O2} = moles of oxygen dissolved in water

N_{H2O} = moles of water

Compared to N_{H2O}, N_{O2} is negligible, so x_{O2} becomes:

$$x_{O2} = \frac{N_{O2}}{N_{H2O}}$$

and, $$N_{H2O} = \frac{W_{H2O}}{M_{H2O}}$$

where, W_{H2O} = Weight of water

M_{H2O} = Molecular weight of water

Now, 1 ml of water at 15°C weighs virtually 1g,

$$\text{so, } N_{H2O} = \frac{3}{18}$$

$$= 0.167$$

$$\therefore N_{O2} = x_{O2} \times N_{H2O}$$

$$= 5.78 \times 10^{-6} \times 0.167$$

$$= 9.6526 \times 10^{-7} \text{ moles}$$

At S.T.P. one mole of any gas occupies 22.414 dm^3 (i.e. litres).

$$\text{Vol. gas dissolved} = 9.6526 \times 10^{-7} \times 22.414$$

$$= 2.1635 \times 10^{-5} \text{ litres}$$

$$= \underline{21.635 \ \mu l}$$

This is the volume of oxygen dissolved in 3 ml of air saturated water at S.T.P. Throughout the experiment the range of volumes of oxygen available was 20.22 µl to 21.42 µl ($\bar{x}$ ±1 S.D. = 20.91 ±0.42 µl) due to slight variations in the atmospheric pressure from day to day.

(2) To convert the results from a percentage to µl mg^{-1} hr^{-1} at S.T.P.

The results obtained from the chart recorder are in terms of percentage oxygen consumed per gill per 15 minute period. They may be converted to µl mg-1hr-1 thus;

(i) Multiply the percentage by 4 to convert results to 'per hour'.

(ii) Divide by the dry weight to obtain the results 'per mg'.

(iii) Multiply by the volume of oxygen available to get µl mg^{-1} hr^{-1} at S.T.P.

STATISTICAL TREATMENT OF RESULTS

The results shown in Tables 5.1 - 5.7 summarize the raw data. Analysis of results, however, was conducted using all the data based on 6 values per treatment per day (2 gills per crayfish). Two way analysis of variance without replication (i.e. using only sample means) was initially conducted and where the results proved significant the analysis was repeated using two way analysis of variance with replication (i.e. utilizing all data points). This latter test provides a more sensitive result and where more than one treatment is being compared against the control it is also possible to detect interaction between treatments. The two way anovar enables examination, in just one statistical test, for, (i) any significant differences between treated and control results, and, (ii) any significant effect with increasing time of exposure to toxicant/increasing external concentration of toxicant, for the treated samples. Only analysis of the Lindane 0.01 p.p.m. and 0.03 p.p.m. results which employed the same control involved examination of more than two sets of samples (all others were simply; treated vs control), and since there was no interaction between the treatments ($P > 0.1$) it was possible to continue the analysis further using least significant difference (see Parker, 1979) and obtain individual results for each treatment.

The use of two way anovar is more sensitive, and preferable to conducting t-tests on each individual plot, which is neither efficient nor proper. However, since examination of Figs. 5.3 - 5.8 indicated that certain points may differ from the controls, although this was not apparent from the anovar, these points were checked using the t-test. All statistical analyses were

conducted using a Wang desktop computer and standard statistical package.

In order to establish whether either size or sex of crayfish significantly affected the rate of oxygen uptake by excised gill tissue, the following two additional statistical tests were conducted.

(i) Within each of; Controls, cadmium treated, Lindane treated, all of the results obtained for males were compared against all of the results for females using the t-test. Individual trials were not treated separately, and if any differences occur they would show up using the pooled data.

(ii) The results for animals of size groups 30 - 32.5 mm, 32.6 - 35 mm, 35.1 - 37.5 mm and 37.5 - 40 mm within each of; controls, cadmium treated, and Lindane treated were compared by means of a one way anovar. The results of the individual sexes were not treated separately, and neither were individual trials.

5(iii) RESULTS

Owing to the large number of animals required to complete this series of experimental trials it was necessary to mix animals of both sexes, and of a relatively large size range (30 - 40 mm). However, no differences were observed between the rates of oxygen consumption of excised gill tissue from males or females in either the controls ($t = 0.7783$, $P > 0.1$), the cadmium treated animals ($t = 0.7741$, $P > 0.1$) or the Lindane treated animals ($t = 1.5632$, $P > 0.1$). Furthermore, no differences were observed between different size groups of animals tested in either of the controls ($F = 2.876$, $P > 0.05$), the cadmium treated animals ($F = 0.7689$, $P > 0.5$) or the Lindane treated animals ($F = -6.18$, $P > 0.5$).

In order to check that the respirometers used were actually recording the rate of tissue oxygen consumption, the equipment was switched on and tested using, (i) water alone, (ii) Gill filaments from a dead crayfish, (iii) the water remaining after the gill tissue had been removed and from which certain constituents may possibly have leached out. No decrease in the level of oxygen in the medium was recorded in any case. Thus it is reasonable to conclude that the results presented below indicate the oxygen consumption of excised crayfish gill tissue, and that all results are comparable, being neither affected by sex nor size of animals used.

The results for crayfish exposed to an increasing concentration of toxicant over a fixed time period are expressed in Tables 5.1 and 5.2, and illustrated in Figs. 5.3 and 5.4 for cadmium and Lindane respectively. Those relating to fixed concentrations of toxicant over an increasing time period are expressed in Tables 5.3 and 5.4, and Figs. 5.5 and 5.6 for cadmium 1 p.p.m. and 10 p.p.m. repsectively, and Tables 5.5, 5.6 and 5.7, for Lindane 0.01 p.p.m., and 0.03 p.p.m. respectively, and Figs. 5.7 (0.02 p.p.m.) and 5.8 (0.01 p.p.m and 0.03 p.p.m.). The results of statistical analysis of each experiment are given in Table 5.8.

CADMIUM

Results of the two way anovar given in Table 5.8 reveal that tissue oxygen consumption of gills excised from crayfish previously exposed to 1 p.p.m. and 10 p.p.m. cadmium are in neither case significantly different from their respective controls, and nor is there any significant effect with increasing time of exposure ($P > 0.05$ in each case). Fig. 5.5, however, shows

an apparent increase in tissue oxygen consumption for crayfish exposed to 1 p.p.m. cadmium for 1 day, but not thereafter. This difference is significant ($t = 2.76$, $P < 0.02$). For crayfish exposed to 10 p.p.m. cadmium there is an apparent decrease in tissue oxygen consumption up to day 3, but not thereafter (Fig. 5.6). These differences also proved to be significant (Day 1, $t = 2.21$, $P < 0.05$; Day 3, $t = 3.40$, $P < 0.01$). In order to compare the results from the 1 p.p.m. trial with those from the 10 p.p.m. trial it was first necessary to compare both sets of controls, since the same control was not used for each trial. There was no significant difference between either set of controls ($P>0.05$) and they did not differ with time ($P > 0.05$, Table 5.8). Hence it was valid to compare the two treatments with each other. Compared to 1 p.p.m., the 10 p.p.m. treatment causes a significant decrease in the rate of tissue oxygen consumption ($P < 0.01$) but no significant differences were apparent with increasing time of exposure to toxicant ($P < 0.05$). This decrease in oxygen consumption with increasing cadmium concentration is also apparent after 3 days exposure to a range of concentrations (Fig. 5.3). The decrease observed is significantly different from the controls ($P < 0.01$), and there is also a significant decrease with increasing concentration ($P < 0.01$).

LINDANE

No significant difference was observed between control and treated tissues after 24 hours exposure to a range of concentrations of Lindane ($P > 0.05$), and neither was there any significant effect of increasing concentration ($P > 0.05$). Analysis of the trial which examined the concentration of 0.02 p.p.m. Lindane

over a period of 9 days also failed to reveal any significant differences either between control and treated tissues ($P > 0.05$), or with increasing time of exposure ($P > 0.05$). The trial employing concentrations of 0.01 p.p.m. and 0.03 p.p.m. Lindane did, however, produce a significant result. There was a significant difference between the treatments and the control ($P < 0.05$), and also a significant effect with increasing time of exposure ($P < 0.05$). Since the same control was used for each concentration of toxicant, the analysis was taken further (using L.S.D. see 5(ii)) to determine if both treatments differed significantly from the control. This proved to be the case (0.01 p.p.m., $t = 22.36$, $P < 0.001$; 0.03 p.p.m., $t = 5.19$ $P < 0.001$). This analysis, however, encompasses all the data involved, and masks the effect which is apparent in Fig. 5.8 for the higher concentration after 12 and 15 days exposure to toxicant. Both concentrations of toxicant cause a decrease in the tissue oxygen consumption, but at 0.03 p.p.m. after 12 days the rate of oxygen consumption has returned to that of the control, and this is also the case after 15 days. A concentration of 0.01 p.p.m. Lindane depresses the rate of tissue oxygen consumption to a significantly greater extent than 0.03 p.p.m. Lindane ($t = 17.16$, $P < 0.01$).

5 (iv) DISCUSSION

Crayfish gill structures and their mode of functioning during osmoregulation and respiration have been described, for example by Fisher (1972), and Burggren *et. al.* (1974). The gross external structure is summarized in Fig. 5.9. All of the different branchia comprising one gill (i.e. from one side of the animal) were used in these experiments. Water flow over the external gill surfaces

is achieved in the intact animal by the generation of a negative pressure from the beating of the scaphognathite. Within each of the branchiae canals direct blood to and from the filaments so that gas and ion exchange may then occur with the water outside the gill. Each filament, being the finest part of the gross structure, is divided longitudinally into two channels by a septum of connective tissue so that blood flow within each filament also may be bidirectional. Oxygenated blood returns from the efferent channel of the filament to the peripheral canal in the main stem of the branchia. The septum, however, is not continuous, and is broken by small evenly spaced orifices (the lacunae) through which deoxygenated blood will pass from the afferent channel into the efferent channel.

The fine structure of the gill filaments of *Astacus pallipes* have been described in detail by Fisher (1972). A cuticle composed of epi-, exo-, and endo-cuticle components, forms the external part of the gill across which communication between blood and water must occur. That part of the gill which communicates directly with the blood consists of a granular basement membrane, and between this and the cuticle are a layer of epithelial cells. The cytoplasm of these cells contain mitochondria and ribosomes, and large numbers of vessicles. There are two basic types of epithelial cell - one, a thin continuous layer underlying the cuticle, and the other, mushroom shaped cells arising from the thin layer. These cells are embedded in connective tissue which comprises 30 - 50% of the total tissue elements and also forms the septum, previously described. The apical surface of the epithelial cells is frequently indented into villous like structures

in order to increase the functional area of the gills. It is the epithelial cells which are active in respiratory gas and ion exchange, and although the cuticle of the gills is chitinous and relatively impermeable to water and ions, pore canals exist, which may reach a density of 4×10^6 mm^{-2}, and which penetrate up to 75% of the cuticular surface. This leads to enhanced diffusion of gasses, and diffusion from the blood is further enhanced by folding of the basal membrane which results in the formation of channels between cells, allowing an extracellular flow of blood closer to the water.

As previously mentioned, all of the branchiae were used in the respiratory studies, whilst T.E.M. studies were confined to an examination of the fine structure of the filaments on both the podobranchiae, and the arthrobranchiae. Regarding respiratory studies, the respiration of *A. pallipes* under aerial and aquatic conditions has been described (Taylor and Wheatley, 1980), and that of other crustaceans has also been examined under abnormal conditions of salinity (*Carcinus maenas*, Taylor *et. al.*, 1977), oxygen tension (*Homarus vulgaris*, Butler *et. al.*, 1978) and in aerial conditions (*C. maenas*, Taylor and Butler, 1978). This study examines the respiration of *A. pallipes* under abnormal conditions after exposure to toxicants. Fingerman and Lago (1957) have examined the endogenous 24 hour rhythmn of oxygen consumption in the crayfish *Orconectes clypeatus* and have found that peaks of oxygen consumption occur corresponding to dusk and dawn (see Part I, 3.3 active feeding periods) with minima around mid-day. It is likely that a similar effect may occur with *A. pallipes*, but the results obtained above should not be significantly affected since all experiments

were conducted at the same time of day (afternoon). Increased rates of respiration have also been observed to occur at higher temperatures (e.g. Halcrow and Boyd, 1967), but since all experiments were conducted at 15°C ± 1°C this will not affect the results presented above. The possibility of seasonal variations in sensitivity to pollutants has been stressed by Holdich (1981) but neither will this affect the results of this experiment since all data excepting 0.01 and 0.03 p.p.m. Lindane was obtained at the same time of year. The exception arose since it was decided to run an additional experiment with Lindane, and it was indeed found that both the control and treated results demonstrated a reduced oxygen consumption compared to the 0.02 p.p.m. trial (see Figs. 5.7, 5.8) which had been conducted earlier in the year (July vs October). This precluded direct comparison of the 0.02 p.p.m. result with 0.01 and 0.03 p.p.m. by means of the two way anovar. A comparison of the 0.01 p.p.m. and 0.03 p.p.m. results was, however, possible and the results have been presented.

Neither sex nor size was found to affect the rate of tissue respiration in either of the treated or control sets of trials. Heit and Fingerman (1977) reported that both of these variables affected survival of crayfish species during acute toxicity tests using mercury, and the effect of size with respect to juvenile vs. adult crayfish has been reported by this author regarding survival (3). In respirometry tests, however, Dickson and Franz (1980) reported that size did not affect the tissue respiration rates of *Procambarus* spp., whilst Andryushchenko (1972) noted differences between adult and juvenile *Leander adspersus* exposed to D.D.T. Excepting different life cycle stages then, it would

appear that size does not affect the rate of tissue oxygen consumption. Regarding sexual differences, Thurberg *et. al.*, (1977) reported no differences in the rates of respiration of male or female *Homarus americanus* exposed to cadmium, and Vernberg *et. al.* (1974) also reported no sexual differences for cold acclimated *Uca pugilator* exposed to mercury, or in any of the control trials conducted. Sexual differences, however, were apparent for warm acclimated crabs.

The effects of a variety of heavy metal pollutants upon the rates of respiration of crustaceans is summarized in Table 5.9. It will be seen that a range of effects, from increased respiration through no effect to decreased respiration, are reported. This is the case when all the metals are considered, or when just cadmium alone is considered. Neither are the effects totally interspecific since for *A. pallipes* the full range of effects has been reported. What appears to be critical, however, is the precise concentration employed. It is the lowest concentrations of metal which cause an increase in the rate of respiration, whilst higher concentrations tend to cause a decrease. Most authors have studied respiration rates *in vitro* using excised gill tissue, whilst studies conducted *in vivo* show a decrease in oxygen consumption for *Paratelphusa jaquemontii* exposed to cadmium (Kulkarni and Kamath, 1980), and no effect for *Carcinus maenas* exposed to the same metal (Collier *et. al.*, 1973). Interestingly in the latter case, excised gill tissue did show a significant decrease in oxygen consumption with increasing concentration of cadmium. No effect was observed with concentrations of lead, and *Orconectes virilis in vivo* (Anderson, 1978).

The longer term studies conducted by this author indicated that 1 p.p.m. cadmium initially elevated the rate of respiration whilst 10 p.p.m. caused a depression. At both concentrations the rate 'recovered' to that of the control animals, and so although the trial employing a range of concentrations indicated that increasing concentrations of cadmium depressed the rate of respiration further, this may only be a short term effect, and at the other concentrations recovery of normal rates of respiration may have occurred with increased time of exposure. At high metal concentrations the decrease in oxygen consumption observed for excised gill tissues has been attributed to the gill tissue pathology (e.g. Collier *et. al.*, 1973; Thurberg *et. al.*, 1973). Nimmo *et. al.* (1977) have shown that high cadmium concentrations cause necrosis of gill lamellae in *Palaemonetes* spp. and conclude that this may lead to disruption of normal respiration. At sublethal concentrations they found that the level of cadmium in the tissues soon became stable "indicating an equilibrated kinetic state in which the gills can function in respiration and physiological ion balance". By contrast this author was unable to show any cellular changes in gill tissue of *A. pallipes* exposed either to high or low concentrations of cadmium during T.E.M. studies (see 1), despite the fact that the gills were the chief site of uptake (4.1). The changes occurring within the gills, however, need not necessarily cause cell death in order to decrease the rate of tissue respiration. The toxic effects of cadmium were discussed in Chapter 3 (3(iv)) and the ways in which metals can disrupt enzyme systems causing loss of respiratory and osmoregulatory function leading to death were described. Thus cadmium in the tissues

may affect respiration in ways more subtle than simply cell death. At low external cadmium concentrations the increased oxygen consumption observed by this author may have occurred when the level of the metal in the tissue (at a stable state, as above) was too low to result in detectable physiological damage. Indeed, the initial increase observed may have resulted from an increased metabolism due to the energetic demands of producing metal binding proteins (see 3(iv), also Köhler and Riisgård, 1982). These would enable the animal to withstand low concentrations of toxicant, and after the initial increase in respiration the gill starts to function normally as the metals become bound, and unable to cause physiological damage.

The trial using 10 p.p.m. cadmium also showed a recovery of the normal rate of respiration by day 6. This concentration is greater than the safe concentration calculated in Chapter 3 (3.52 p.p.m.) and although no apparent cellular damage is caused at this level, physiological damage probably occurs. The recovery of the tissues to normal respiration rates is thus more difficult to explain, although in the absence of cell death it is possible, and seems probable, that physiological adaptation occurs. Tests conducted *in vivo* found that crayfish exposed to lead (0.5 - 2.0 p.p.m.) showed no statistically significant decrease in their rates of respiration, and decreased gill efficiency due to the lead was compensated for by an increase in the ventilation volume (Anderson, 1978). This indicates that coupled with physiological adaptation, crayfish are reasonably well adapted to be able to survive sublethal concentrations of heavy metals. Anderson (1978) also states that at ecdysis the external surfaces of the gills

are sloughed off, and a large part of the lead present is also removed. Survival then, could be for significant periods in polluted water, and it also seems that short term adaptation to higher concentrations may occur.

Table 5.10 summarizes the effects of various pesticides on the rates of respiration of crustaceans and insects. Lindane increases the rate of respiration of insects (Demozay and Marechal, 1972c), but decreases that of the crustacean *A. pallipes* (present study). Other organochlorine insecticides tested on various crustaceans also caused a decrease in oxygen consumption except for Chlordane at the lowest concentration test (1 p.p.m.), when it caused an increase (Hobbs and Hall, 1975). Malathion, an organophosphate pesticide also caused a decrease in oxygen consumption. The increase in oxygen consumption of the crayfish *P.clarkii* exposed to 1 p.p.m. chlordane has also been observed in shrimp (*Leander adspersus* - Andryushchenko, 1972) exposed to very low concentrations of D.D.T., although higher concentrations cause a decrease. It has been suggested that the increase observed results from mobilization of the bodies' reserves in order to protect the normal course of its vital processes, whilst at higher concentrations the toxic effect cannot be withstood for any length of time resulting in a decreased oxygen consumption. Kulkarni and Kamath (1980) suggest that low concentrations of toxicant are stimulatory in effect and at slightly higher concentrations enzyme systems are activated. At higher concentrations still, they suggest that a reduction in blood pigment transport may explain reduced respiration rates, and the effect of insecticides to stimulate or depress respiratory rates dependent upon concentration has

also been noted by Sigmon (1979).

Lindane is toxic to *A. pallipes*, and its mode of action, which involves the disruption of enzyme activity necessary for respiration, has been discussed (3(iv)). The longer term experiments all used concentrations of toxicant which were below the safe level reported earlier (3(iv), 0.04 p.p.m.) and yet significant effects of the toxicant were apparent upon the rates of respiration of excised gill tissue. No stimulatory effect was observed, and if this were to occur, then it follows from the preceding arguments that concentrations of even less than 0.01 p.p.m. should be studied. Contrary to these arguments, however, recovery of the rate of respiration of animals exposed to 0.03 p.p.m. occurred after 12 days exposure to toxicant. A slight depression in the rate of respiration of gills from crayfish exposed to 0.02 p.p.m., which occurred initially (Fig. 5.7, not statistically significant) also recovered to the normal rate. Only the animals at 0.01 p.p.m. continued to exhibit a decreased oxygen consumption. It is suggested that the above arguments for higher concentrations causing decreased oxygen consumption arise from severe physiological damage. In *A. pallipes* no morphological damage of gills was observed through exposure to Lindane during T.E.M. studies, and the sublethal concentrations used in these experiments probably caused a decreased oxygen consumption due to interference by Lindane with enzymes necessary for respiration (3(iv)). Recovery occurred due to active processes which translocate the Lindane from the gills to the hepatopancreas, where it is possible that some detoxification may occur. This translocation was noted in chapter 4.2. The very lowest concentrations used, however, may not cause sufficient

build up of Lindane in the gills to activate its translocation, and yet sufficient concentrations exist to affect respiratory enzymes. Thus at 0.01 p.p.m. the rate of respiration is depressed below that of 0.03 p.p.m. Further evidence that recovery at low levels of exposure may occur is that at 0.03 p.p.m. 35% of the crayfish showed loss of balance and were on their backs after 24 hours, 50% after 48 hours, 14% after 72 hours, and thereafter all animals had regained their balance. Translocation of the toxicant to the hepatopancreas and its subsequent detoxification (although there is no evidence for an enzyme capable of doing this, see 3(iv)) or excretion at a greater, or equivalent, rate to uptake (see 4.2) may explain this.

After 3 days exposure to toxicant the 0.01 p.p.m. result was the same as the control, 0.02 p.p.m. was depressed and 0.03 p.p.m. was increased, though none were significantly different. The observed depression was only observed to occur from day 6 onwards, and it may be that at these very low concentrations sufficient Lindane had not been taken up by the gills to cause any physiological damage until day 6. 0.01 p.p.m. it is suggested was insufficient to activate relocation, 0.02 p.p.m. was sufficient and relocation occurred fast enough to prevent any change in the rate of respiration, whilst 0.03 p.p.m. caused a depression until the translocation processes were sufficiently activated to cope with the higher concentration. Similar recovery was recorded in crabs with respect to the production of Na,K-ATPase after a period of exposure to D.D.T. (see 3(iv)). That no effect was observed with increasing concentration after 24 hours is probably due to the fact that insufficient Lindane had been taken up by

the gills in this time period. Evidently, however, sufficient enters the animal by either the oral route or across the gills, at the higher concentrations (0.20 p.p.m.), to affect the nervous system resulting in the loss of balance of all animals at this concentration. Fig. 5.4 indicates some depression in the rate of oxygen consumption for this trial, although this did not prove significant.

In conclusion it is apparent that both cadmium and Lindane affect the rate of respiration of excised gill tissue of *A. pallipes*, and cause a depression. The significant point to note, however, is that concentrations of both toxicants which are less than those considered safe for *A. pallipes*(3(iv)) still cause a measurable effect when respiration is monitored, although recovery to normal oxygen consumption occurs with increasing time of exposure. This is despite the continued uptake of toxicant as demonstrated in chapter 4 (4.1, 4.2).

TABLES 5.1 - 5.7 TO SHOW THE TISSUE OXYGEN CONSUMPTION OF EXCISED CRAYFISH GILL FILAMENTS AFTER EXPOSURE TO TOXICANTS UNDER VARIOUS REGIMES

5.1 CADMIUM, 1 p.p.m. - 50 p.p.m., 3 days

TREATMENT	CONCENTRATION OF TOXICANT (p.p.m.)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	1.0	2.40-3.76	3.24	0.23
Control	-	2.50-4.37	3.24	0.36
Treated	5.0	2.53-3.62	3.11	0.25
Control	-	3.35-5.38	3.92	0.32
Treated	10.0	1.09-2.60	1.68	0.21
Control	-	1.92-3.89	2.95	0.31
Treated	25.0 *	1.64-2.63	2.13	0.49
Control	-	3.35-5.38	3.92	0.32
Treated	50.0	0.83-1.53	1.14	0.07
Control	-	1.47-2.23	1.89	0.11

* NB This result relates to only 2 sets of data, due to the increased susceptibility of Markfield stock to cadmium ions (see 5(ii)).

5.2 LINDANE, 0.02 p.p.m. - 0.20 p.p.m., 1 day

TREATMENT	CONCENTRATION OF TOXICANT (p.p.m.)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	0.02	2.01-3.08	2.53	0.16
Control	-	1.70-3.68	2.68	0.26
Treated	0.05	1.70-2.49	2.02	0.17
Treated	0.15	1.57-2.10	1.74	0.08
Treated	0.20	1.51-2.54	1.99	0.18
Control	-	1.51-3.67	2.21	0.34

5.3 CADMIUM, 1 p.p.m., 1 - 9 days

TREATMENT	TIME OF EXPOSURE TO TOXICANT(DAYS)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	1	3.05-4.33	3.56	0.22
Control		1.78-3.60	2.63	0.25
Treated	3	2.40-3.76	3.24	0.23
Control		2.50-4.37	3.24	0.36
Treated	6	2.33-3.36	2.57	0.21
Control		1.66-3.93	2.44	0.31
Treated	9	2.18-3.10	2.68	0.16
Control		1.28-4.63	2.95	0.53

5.4 CADMIUM, 10 p.p.m., 1 - 9 days

TREATMENT	TIME OF EXPOSURE TO TOXICANT(DAYS)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	1	1.41-2.65	2.22	0.20
Control		2.90-3.11	3.00	0.11
Treated	3	1.09-2.60	1.68	0.21
Control		1.92-3.89	2.95	0.31
Treated	6	1.12-2.59	1.86	0.26
Control		1.43-2.28	1.87	0.22
Treated	9	1.24-1.88	1.56	0.09
Control		1.02-1.80	1.50	0.18

5.5 LINDANE, 0.01 p.p.m., 3 - 12 days

TREATMENT	TIME OF EXPOSURE TO TOXICANT(DAYS)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	3	1.02-3.31	1.74	0.34
Control		0.97-2.34	1.74	0.20
Treated	6	0.75-1.46	1.15	0.10
Control		1.04-3.04	1.80	0.31
Treated	9	0.91-1.42	1.23	0.08
Control		1.22-3.53	2.09	0.32
Treated	12	1.00-2.03	1.54	0.16
Control		1.64-3.90	2.40	0.33

5.6 LINDANE, 0.02 p.p.m., 1 - 9 days

TREATMENT	TIME OF EXPOSURE TO TOXICANT(DAYS)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated	1	2.01-3.08	2.53	0.16
Control		1.78-3.60	2.63	0.25
Treated	3	2.40-4.01	2.99	0.22
Control		2.50-4.37	3.24	0.36
Treated	6	2.63-3.68	3.03	0.15
Control		1.66-3.93	2.44	0.31
Treated	9	2.15-3.67	2.97	0.24
Control		1.28-4.63	2.95	0.53

5.7 LINDANE, 0.03 p.p.m., 3 - 15 days

TREATMENT	TIME OF EXPOSURE TO TOXICANT(DAYS)	TISSUE OXYGEN CONSUMPTION, $\mu l mg^{-1} hr^{-1}$		
		RANGE	MEAN	S.E.
Treated Control	3	1.33-2.38 0.97-2.34	1.92 1.74	0.20 0.20
Treated Control	6	1.24-2.28 1.04-3.04	1.63 1.80	0.17 0.31
Treated Control	9	1.01-2.20 1.22-3.53	1.46 2.09	0.17 0.32
Treated Control	12	1.40-3.21 1.64-3.90	2.44 2.40	0.27 0.33
Treated Control	15*	2.24-2.94 2.00-3.23	2.59 2.60	0.35 0.25

* NB. This result relates to only 2 sets of data, and utilized extra animals placed at this concentration in case of mortalities during the experiment. None occurred. Day 15 was examined to check the Day 12 result.

TABLE 5.8 A SUMMARY OF THE STATISTICAL TREATMENT OF THE RESULTS EXPRESSED IN TABLES 5.1 - 5.7 USING A TWO WAY ANOVAR

REGIME EMPLOYED	TOXICANT vs CONTROL			INCREASING TIME OR CONCENTRATION		
	F	df	P	F	df	P
5.1 Increasing Cd concentration	70.58	1,47	<0.01	70.10	3,47	<0.01
5.2 Increasing γBHC concentration	2.43	1,47	>0.05	2.25	3,47	>0.05
5.3 1 p.p.m. Cd, increasing time	0.58	1,7	>0.05	1.58	3,7	>0.05
5.4 10 p.p.m. Cd, increasing time	2.45	1,7	>0.05	2.24	3,7	>0.05
5.6 0.02 p.p.m. γBHC, increasing time	1.67	1,7	>0.05	0.13	3,7	>0.05
5.5 + 5.7 0.01 + 0.03 p.p.m., γBHC, increasing time	5.78	2,6	<0.05	3.97	3,6	<0.05
5.3 + 5.4 Control of control	1.65	1,7	>0.05	1.47	3,7	>0.05
5.3 + 5.4 1 p.p.m. of 10 p.p.m.	42.54	1,7	<0.01	3.59	3,7	>0.05

TABLE 5.9 A SUMMARY OF THE EFFECTS OF HEAVY METALS ON THE RATE OF RESPIRATION OF CRUSTACEANS

ORGANISM	METAL (CONC.)	TISSUES	O_2 INCREASE/ DECREASED?	SEX/SIZE DIFFERENCE	NOTES	REFERENCE
Austropotamobius pallipes	Cd (1 p.p.m.)	Ex. gill	No effect	No,sex/size	Initial increase, recovery to control values by day 3	Author
A. pallipes	Cd (10 p.p.m.)	Ex. gill	No effect	No,sex/size	Initial decrease, recovery to control values by day 6	Author
A. pallipes	Cd (1-50 p.p.m.)	Ex. gill	Decrease	No,sex/size	Day 3. Decrease with increasing concentration.	Author
Eurypanopeus depressus	Cd (1-48 p.p.m.)	*In vivo*	No effect	-		Collier *et.al.*, 1973
E. depressus	Cd (1-48 p.p.m.)	Ex. gill	Decrease	-	Decrease with increasing concentration	Collier *et.al.*, 1973
Carcinus maenas	Cd (1-8 p.p.m.)	Ex. gill	Decrease	-	48 hr. exposure. Loss of osmoregulatory control	Thurberg *et.al.*, 1973
Cancer irroratus	Cd (1-8 p.p.m.)	Ex. gill	Decrease		48 hr. exposure. Loss of osmoregulatory control	Thurberg *et.al.*, 1973
Homarus americanus	Cd (3-6 p.p.b.)	Ex. gill	Increase	No, sex	30 days exposure	Thurberg *et.al.*, 1977
Paratelphusa jaquemontii	Cd (5 p.p.m.)	*In vivo*	Decrease	-		Kulkarni&Kamath,1980
C. maenas	Cu (0.1 m eq)	Ex. tissues	Decrease	-	Heart and gill tissues most severely affected	Kerkut & Munday,1962
C. maenas	Cu (2-5 p.p.m.)	Ex. gill	No effect	-	48 hr. exposure. Loss of osmoregulatory control	Thurberg *et.al.*, 1973
C. irroratus	Cu (2.5 p.p.m.)	Ex. gill	No effect	-	48 hr. exposure. Loss of osmoregulatory control	Thurberg *et.al.*, 1973
Uca pugilator	Hg (0.18 p.p.m.)	Ex. gill	Decreased	Yes, sex	7 days exposure. Sex differences only warm aclimatised animals.	Vernberg *et.al.*, 1974
Orconectes virilis	Pb (0.5-2 p.p.m.)	*In vivo*	Decreased	-	Statistically insignificant result	Anderson, 1978
Procambarus spp.	None	Ex. gill	-	No, size		Dickson & Franz,1980

TABLE 5.10 A SUMMARY OF THE EFFECTS OF PESTICIDES ON THE RATES OF RESPIRATION OF SOME ORGANISMS

ORGANISM	PESTICIDE (CONC.)	TISSUE	O_2 INCREASE/ DECREASED?	SEX/SIZE DIFFERENCE	NOTES	REFERENCE
Austropotamobius pallipes	Lindane (0.01 p.p.m.)	Ex. gill	Decreased	No,sex/size	12 days exposure.Decrease greater than 0.03 p.p.m.	Author
A. pallipes	Lindane (0.02 p.p.m.)	Ex. gill	No effect	No,sex/size	9 days exposure.	Author
A. pallipes	Lindane (0.03 p.p.m.)	Ex. gill	Decreased	No,sex/size	15 days exposure. Recovery to control values day 12	Author
A. pallipes	Lindane (0.02-0.2 p.p.m.)	Ex. gill	No effect	No,sex/size	24 hrs. exposure.	Author
'Insects'	Lindane	-	Increased	-	Review. terrestrial insects.	Demozay & Marechal 1972c
Procambarus clarkii	Chlordane (1 p.p.m.)	-	Increased	-	Organo-Cl. O_2 cons. v. high.	Hobbs and Hall,1975
P. clarkii	Chlordane (2 p.p.m.)	-	Decreased	-	O_2 cons. v. low.	Hobbs and Hall,1975
P. clarkii	Chlordane (4,8 p.p.m.)	-	Decreased	-	O_2 cons. decreased but greater than 2 p.p.m.	Hobbs and Hall,1975
'Crayfish'	D.D.T. (0.5 p.p.m.)	-	Decreased	-	Organo-Cl.	Freeman& Hall, 1970
Leander adspersus	D.D.T. (0.1-0.0001 p.p.m.)	*In vivo*	Decreased	Yes, size	Juveniles more sensitive	Andryushchenko, 1972
Paratelphusa jaquemontii	Endrin (0.1 p.p.m.)	*In vivo*	Decreased	-	Organo-Cl.	Kulkarni & Kamath, 1980
P. jaquemontii	Malathion (1 p.p.m.)	*In vivo*	Decreased	-	Organo-PO_4	Kulkarni & Kamath, 1980

FIG. 5.1 THE RANK OXYGEN ELECTRODE

The Rank oxygen electrode consists of a perspex experimental chamber (1) with an outer jacket (2) allowing cooling water to be circulated maintaining a constant temperature. This unit fits into a recessed perspex holder which houses a platinum/silver-silver chloride electrode (3), which is saturated with molar potassium chloride and covered with a teflon membrane held in place by a rubber ring (4). The whole unit sits on a magnetic stirrer (5) such that a magnetic 'flea' ensures complete circulation of the medium above the electrode. To prevent gaseous exchange with the atmosphere a perspex screw (6) with rubber sealing ring (7) is screwed down to the surface of the medium. A fine drilled hole (8) enables the escape of trapped air and allows the injection of small quantities of toxicant if acute tests are being conducted. Oxygen in the medium diffuses across the teflon membrane and is reduced at the platinum surface;

$$O_2 + 2E + 2H^+ \longrightarrow H_2O_2$$

$$H_2O_2 + 2E + 2H^+ \longrightarrow 2H_2O$$

The platinum electrode is polarized at -0.6V with respect to the silver chloride electrode. Hence a current flows which is proportional to the activity (partial pressure) of oxygen in solution. The electrode is connected through a potential divider to a chart recorder.

Fig.5.1

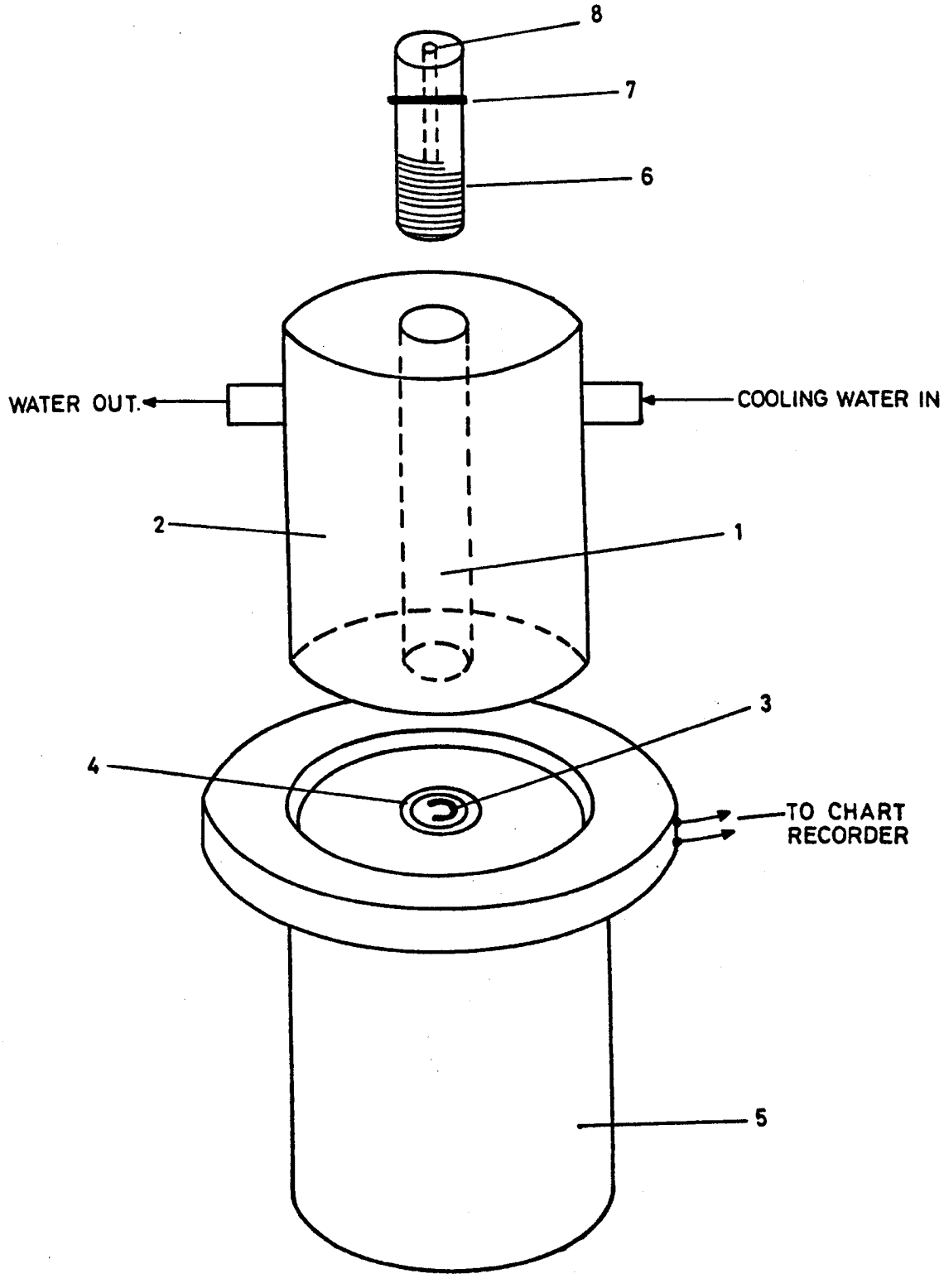


FIG. 5.2 CALIBRATION OF THE RANK OXYGEN ELECTRODE

Air saturated water causes a full scale deflection on the chart recorder (Point A), and represents the situation with 100% available oxygen. Nitrogen bubbled through the water causes the level of oxygen to fall until at point B, when a steady state has been reached, the chart recorder represents a situation with 0% available oxygen. The vertical distance A-B represents 0-100% available oxygen, and the proportion of this distance moved when gill tissues take up oxygen from the water, enables calculation of the percentage consumption of the available oxygen by the tissue to be calculated.

FIG. 5.2 DIAGRAMATIC REPRESENTATION OF A CHART RECORDER TRACE DURING CALIBRATION

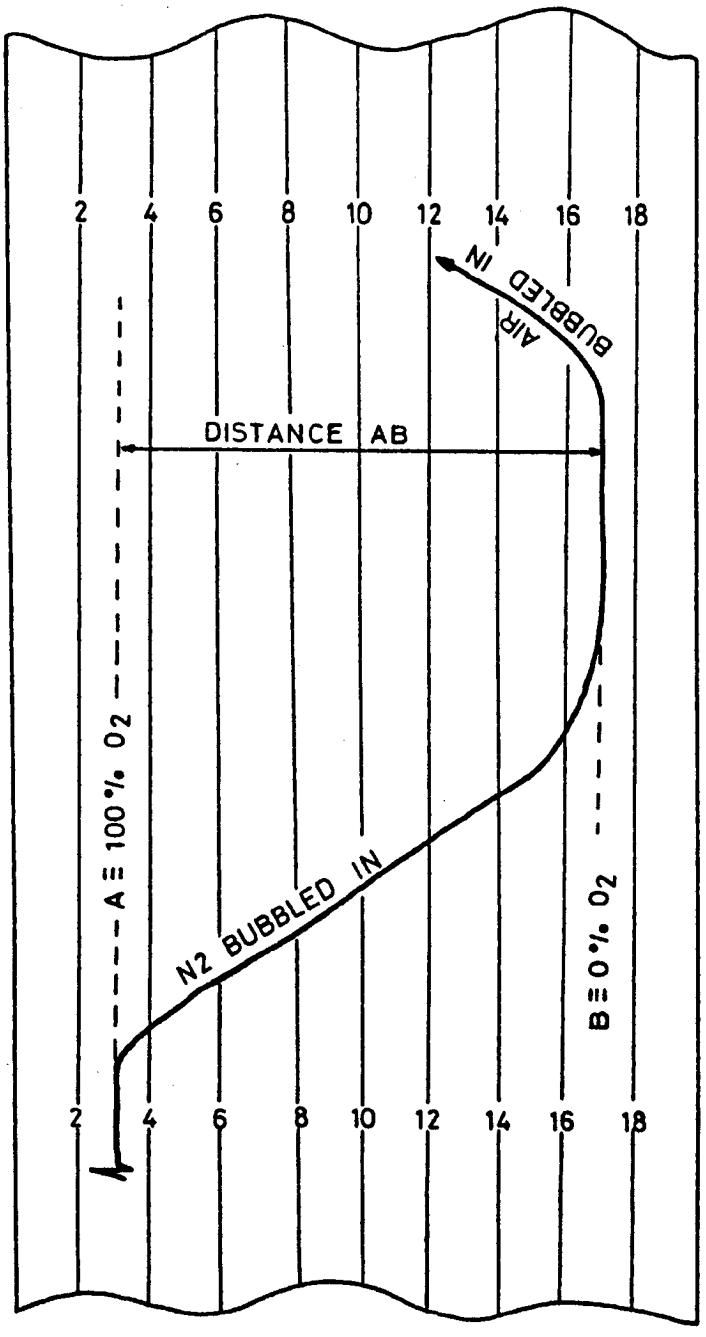
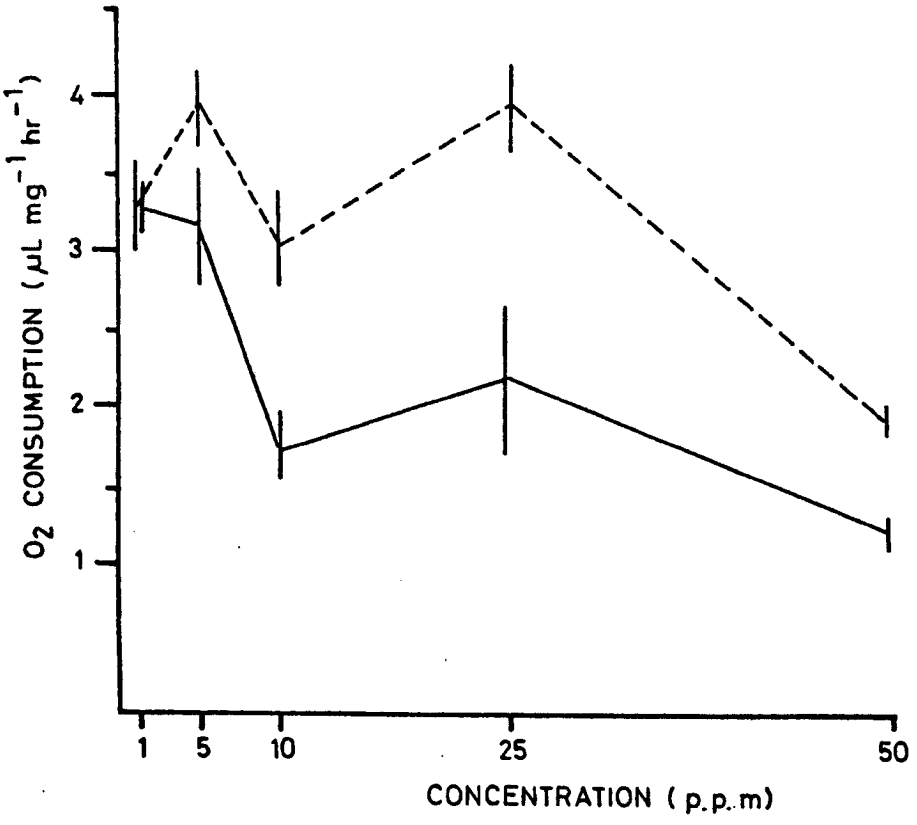


FIG. 5.3 - 5.8 TO SHOW GILL TISSUE OXYGEN CONSUMPTION UNDER VARIOUS TIME/CONCENTRATION REGIMES OF EXPOSURE TO TOXICANT.

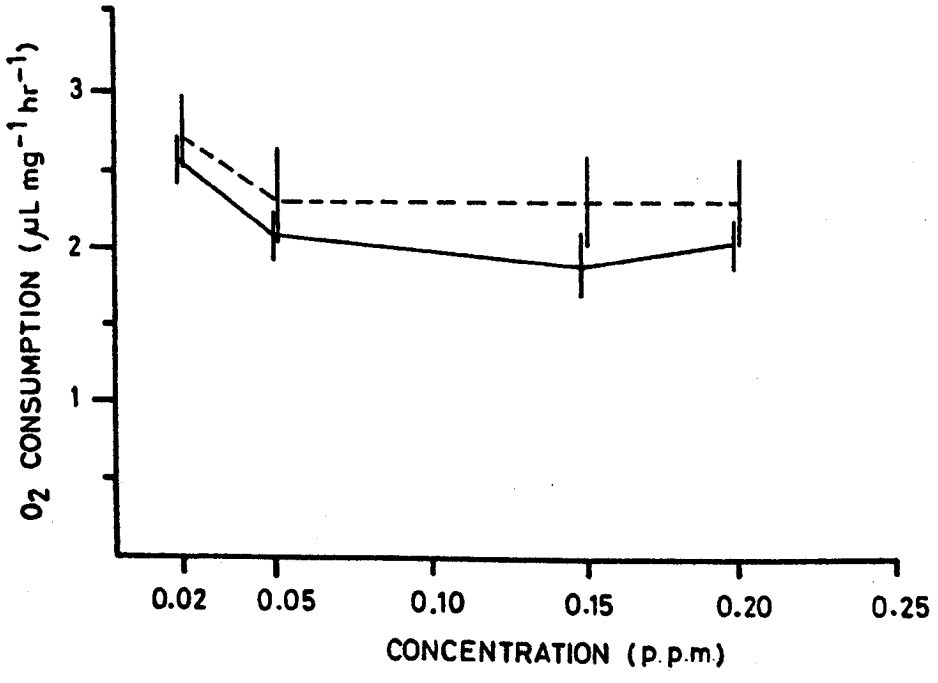
These figures show the rate of respiration of excised gill tissue after various treatments, expressed as μl O_2 consumed per mg dry weight of tissue per hr. The results of the treated tissues are shown as a solid line, whilst the controls are shown as a broken line. The standard errors of each data point are also indicated in the figures.

FIG. 5.3, 5.4 TO SHOW THE TISSUE OXYGEN CONSUMPTION OF EXCISED CRAYFISH GILLS AFTER EXPOSURE FOR 3 DAYS, AND 1 DAY, TO INCREASING EXTERNAL CONCENTRATIONS OF CADMIUM (5.3) AND LINDANE (5.4) RESPECTIVEY

5.3 CADMIUM

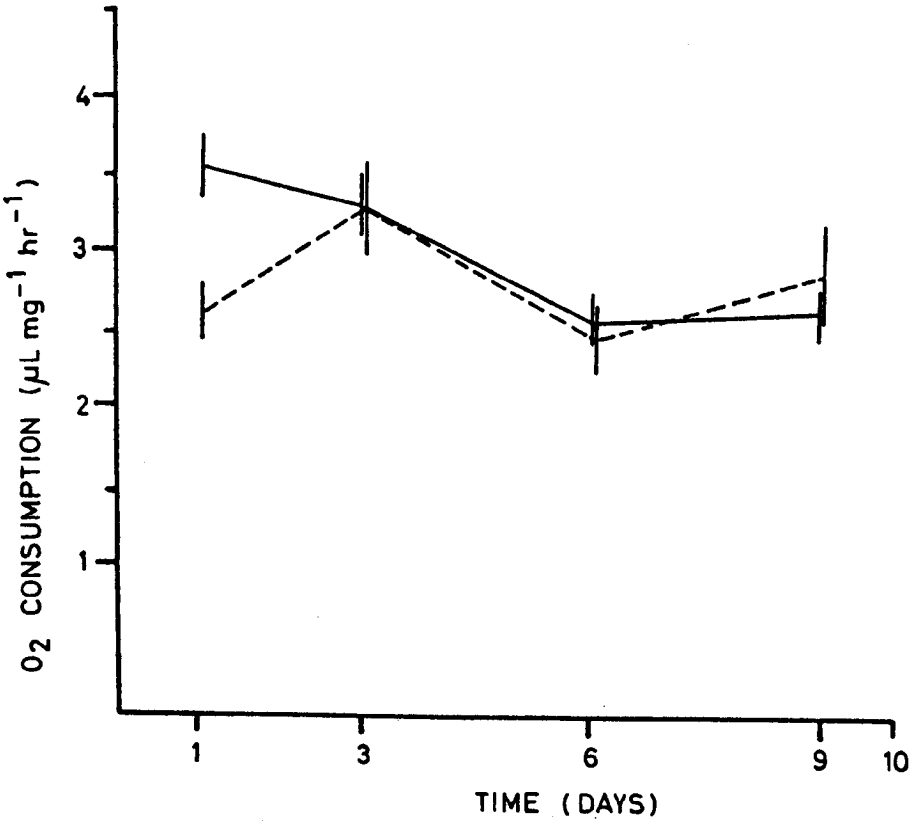


5.4 LINDANE

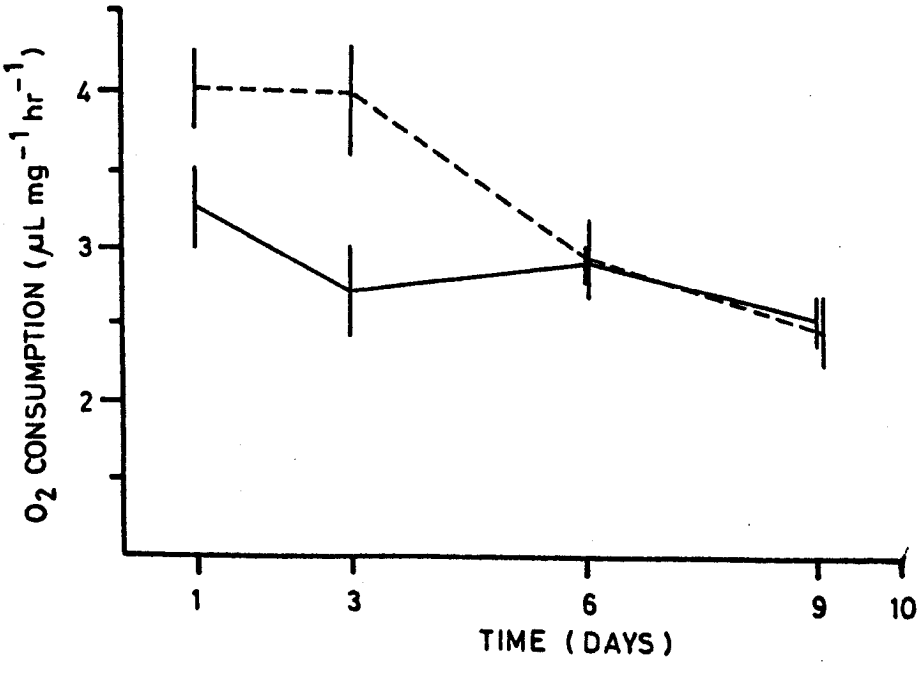


FIGS. 5.5, 5.6 TO SHOW THE TISSUE OXYGEN CONSUMPTION OF EXCISED CRAYFISH GILLS AFTER EXPOSURE FOR UP TO 9 DAYS TO 1 PPM (5.5) AND 10 PPM (5.6) CADMIUM IONS

5.5 1ppm

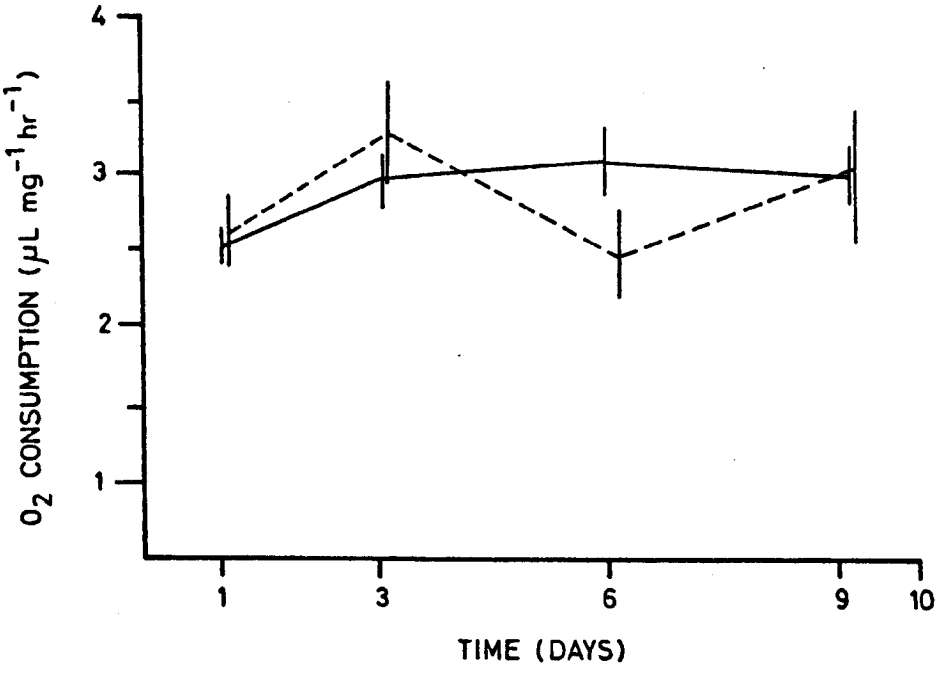


5.6. 10 ppm.



FIGS. 5.7, 5.8 TO SHOW THE TISSUE OXYGEN CONSUMPTION OF EXCISED CRAYFISH GILLS AFTER EXPOSURE FOR UP TO 15 DAYS TO 0.02 PPM (5.7) AND 0.01, 0.03 PPM (5.8) LINDANE

5.7 0.02 ppm.



5.8 0.01, 0.03 ppm.

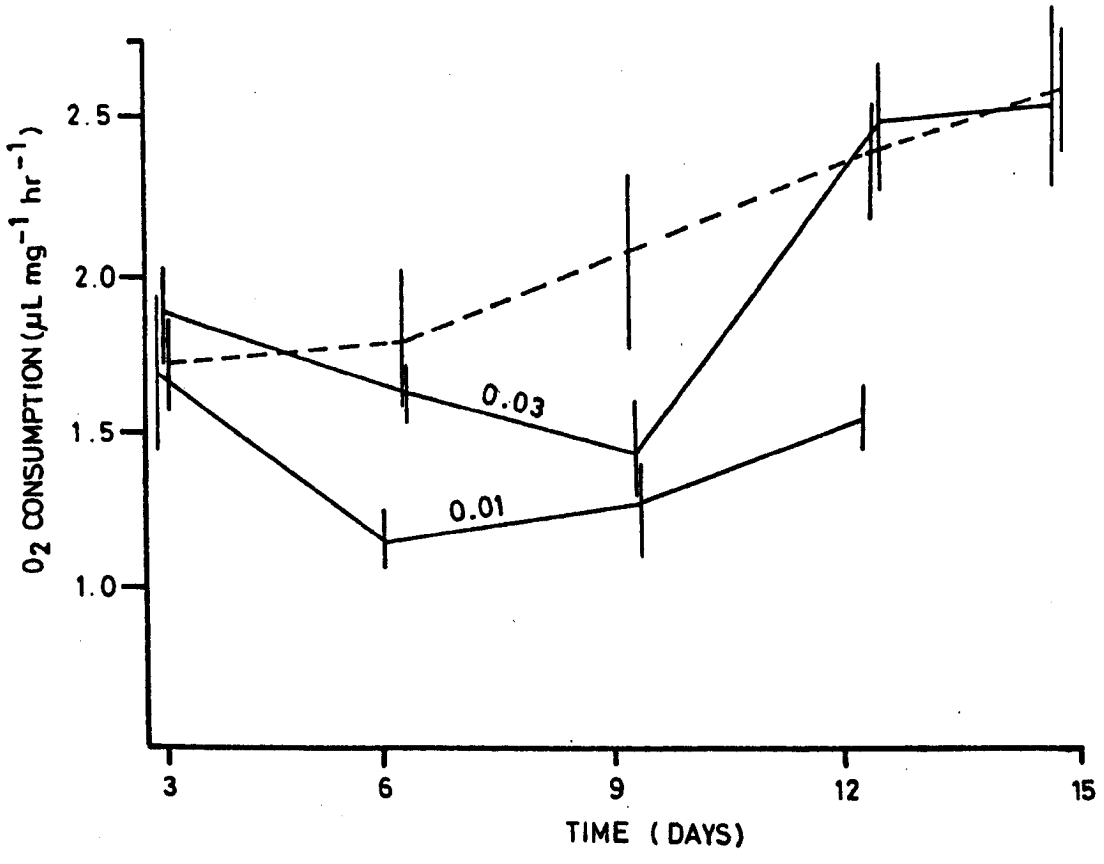
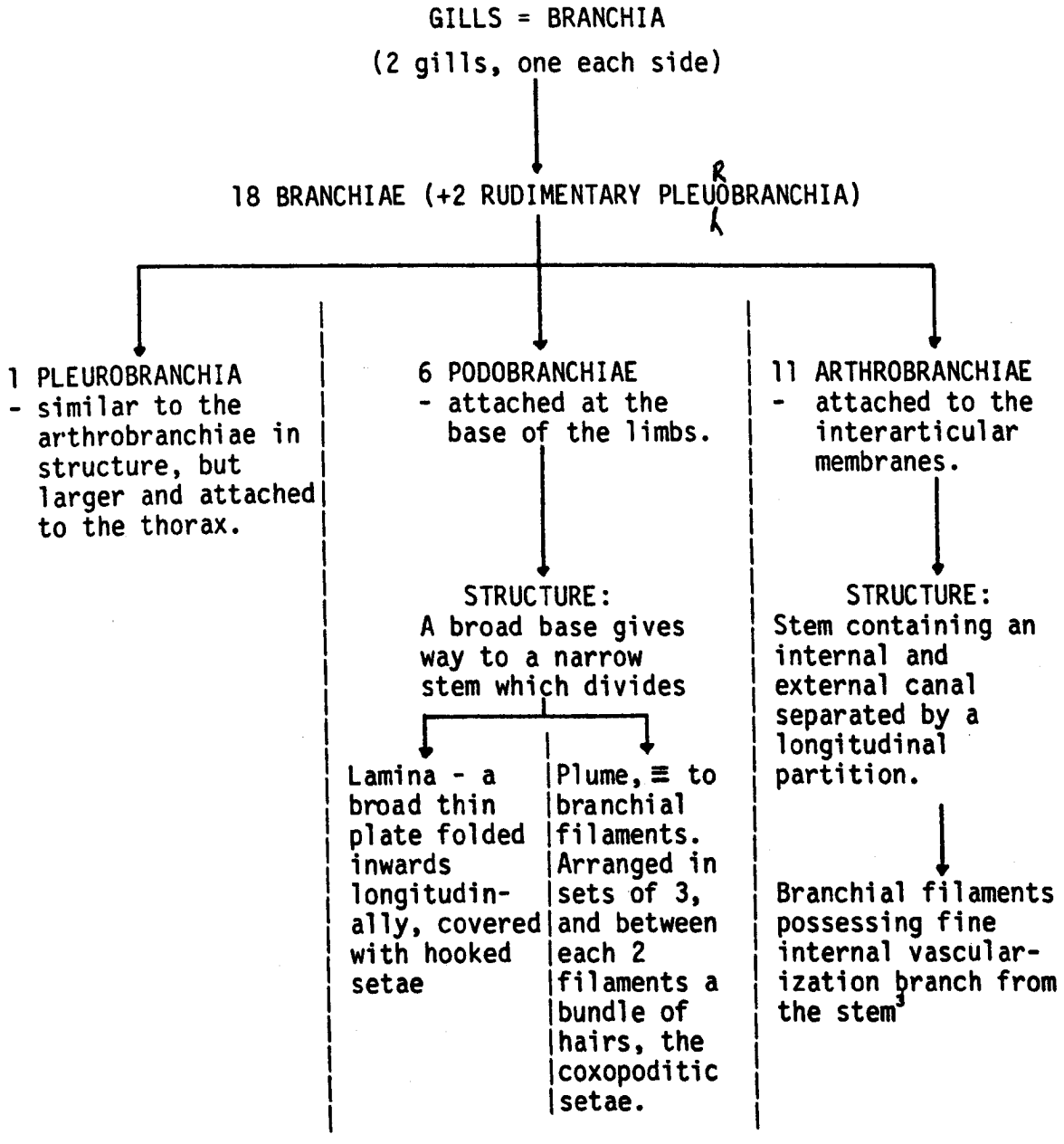


FIG. 5.9 THE GROSS ANATOMY OF CRAYFISH GILLS



CHAPTER 6

GENERAL DISCUSSION TO PART II

Heavy metals and pesticides are both potential pollutants which have been implicated in the disappearance of populations of *A. pallipes* in Britain. The effects of both cadmium and Lindane, two toxicants found in rivers in the Severn-Trent region, have been investigated in relation to their effect upon *A. pallipes*. Their toxic effects were described, and lethal concentrations established (Chapter 3). From these 'safe' concentrations were derived according to the formula derived by Doudoroff *et.al.*(1951).

In Chapter 5 it was established that sublethal effects of these toxicants could be observed at concentrations below those described as 'safe'. Chapter 4 showed that both pollutants could be accumulated from the environment, but that only cadmium is of potential concern when present at low concentrations because it continues to accumulate in the tissues with increasing time of exposure. Thus 'safe' concentrations in the environment could potentially reach lethal levels in the animal and may not accurately be described as 'sublethal'. Lindane, on the other hand, was eliminated at the same time as it was being taken up, so 'safe' concentrations would not be of major concern, and safely may be termed as 'sublethal' concentrations.

This argument does not take into account any effects of these pollutants upon reproduction, behaviour, or potential increased susceptibility at moulting. Studies of the chronic effects of these pollutants in relation to such factors were not conducted but could usefully form the basis for further research. Other suggestions have also been made in Section II as to how the research

programme initiated by this author might be continued.

Chapter 4 looked also at the sites of uptake of cadmium and Lindane, and the point was made that this was necessary if *A. pallipes* were to be a potential food resource. Although accumulation from the environment does occur most of the cadmium is found in the gills, and most of the Lindane in the hepatopancreas. Neither organ is consumed, and the muscle, that part which is eaten, was seen to accumulate only trace amounts of either toxicant. Hence, should crayfish be caught from water bodies sub-lethally polluted with these toxicants, there is little danger of transmission of the toxicant to man. Nevertheless, consumption of crayfish from waters known to be polluted should not be encouraged.

The respiration of *A. pallipes* was shown to be affected by sublethal levels of both cadmium and Lindane (Chapter 5), although the gill tissue monitored was able to recover normal rates of respiration with time, and it was also reported that increased ventilation of the gills could occur to maintain normal conditions. The evidence suggested that low levels of either toxicant could be tolerated by adult crayfish, but an increased sensitivity of juveniles was demonstrated (Chapter 3).

All of the previous discussion in Part II has centred upon the individual effects of cadmium or Lindane, whilst all other parameters of the environment were maintained at as near to optimum conditions as possible. This derives from the nature of the experiments which were designed specifically to examine the effects of these toxicants. However, it must be realised that in the natural situation, and particularly one where pollution occurs, conditions will not always be optimum, and there may be more

than one pollutant present in the environment. Thus synergistic effects may occur with factors such as decreased oxygen levels, increased temperatures, high or low pH, and reduced water hardness, for example, acting to enhance the toxic effects of either cadmium or Lindane. Where two or more pollutants occur, even cadmium and Lindane together, then they may act synergistically. Thus the results presented may not directly be transferred to the field situation. The possibility of further research thus once again presents itself.

In both study areas (the East branch of the Leen, and Markfield Quarry), heavy metals were reported at low concentrations, whilst greater concentrations occurred in the Leen after the confluence of the two branches. Crayfish seldom occur beyond the confluence (Part I, 3.3), and no single factor of the water quality appears to be responsible for this. However, it appears that this situation represents an example of synergism since the increased heavy metal concentrations, which although when considered separately would not constitute toxic levels, act together to exclude *A. pallipes*.

Multiple environmental effects have been studied, particularly in the marine situation (e.g. Vernberg *et. al.*, 1974), and the effects of increased temperature were briefly mentioned in this study. Sprague (1970) has discussed the effects of combining two or more pollutants and their interactive effects, whilst Engel *et. al.*, (1981) have reviewed the environmental parameters which might affect the uptake and toxicity of heavy metals. One of particular relevance to cadmium relates to the water hardness, and it is found that cadmium becomes relatively less toxic in

waters with high calcium concentrations, and its uptake into the haemolymph of crustaceans has been shown to be strongly inversely related to the calcium concentration of the external medium (Wright, 1980). Another factor which may affect the toxicity of heavy metals is the form in which they are available to the organism. In the natural situation chelation with natural or anthropogenic organic ligands may occur, dramatically reducing the toxicity of the metals, and altering the dynamics of their uptake (Ray *et. al.*, 1979b). Similarly insecticides might tend to occur in sediments more than in the aquatic environment itself, thus reducing their potential toxicity. Both cadmium and Lindane can, however, be accumulated from food and sediments (4.1, 4.2).

GENERAL CONCLUSION

This thesis has been presented in two parts. Part I dealt with the ecology of *A. pallipes* in the Midlands, and compared a population from a fast flowing shallow stream (the River Leen), with that of a standing, and relatively deep water body (Markfield Quarry). Part II examined the effects of two potential pollutants (cadmium and Lindane) upon *A. pallipes* obtained from Midlands populations. The purpose of this final section is to draw the reader's attention to the fact that each part of the thesis bears direct relevance to the other, by a brief recapitulation of the conclusions that bear on the common theme.

The thesis of Part I was that certain aspects of the ecology of *A. pallipes* need to be studied in order to form a baseline survey for reference in the face of suspected population decline. This may arise from accidential introductions of the crayfish plague, competition from exotic species, or from pollution of the waterways. The information required includes estimates of the natural population sizes that may be expected in particular types of unpolluted natural waters, together with studies of growth patterns and fecundity related to population density and food supply (Part I). Without such baseline surveys it would be all too easy to attribute altered growth and fecundity wrongly to the effects of competition or pollution. This study was able to demonstrate that crayfish populations in the Leen are apparently very susceptible to small changes in the total level of pollutants between the upper and lower reaches of the river, but more work of the type undertaken in Part II would be needed to identify

substances that are especially important. Furthermore, it has been suggested that such work should be extended to look at the chronic effects of sublethal levels of pollutants (on growth, moulting, or fecundity) so as to provide a better basis for distinguishing these from natural fluctuations in the same parameters.

In Part I the possibility of exploiting *A. pallipes* as a food resource was also discussed. In this connection studies of the growth and relative growth of the body parts of *A. pallipes* under natural conditions were conducted, and the exploitable proportion of a population was established. Part II examined the uptake of toxicants into the tissues of *A. pallipes* and established that the dangers of transmission of toxicant to man were minimal, since the muscle tissue accumulated very little. Accumulation was observed to occur in other tissues (the gills and hepatopancreas), so danger of contaminating the cooking water which may be used as a stock, does exist. This implies that for crayfish of dubious origin, only the tails and claws should be cooked, whilst the main body carcass should be discarded. This should be easily provided for in the arrangements for marketing the produce, although it was not suggested that *A. pallipes* would form the basis of a large scale commercial venture. The dangers of such accumulation occurring are real, since it was established that adult crayfish possessed mechanisms enabling them to store a substantial burden of both heavy metal and insecticide pollutants in, to them, a non-toxic form. They are thus able to survive in polluted environments, although possibly not reproduce due to greater juvenile susceptibility.

In conclusion, it is apparent that the sort of baseline study made available in this thesis has come just in time. Now that evidence for the crayfish plague within British waters appears to exist, and reports of the introductions of exotic species into natural waterways are increasing, the value of the findings of Part I of this thesis are enhanced. Those of Part II form a unique study because they make specific information available for *A.pallipes* which until now has been lacking.

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